Robotic Training for Motor Rehabilitation after Complete Spinal Cord Injury

Thesis by

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Abstract

The spinal cord circuits have a great degree of automaticity and plasticity. They are able to generate complex locomotor patterns such as stepping and scratching even without input from supraspinal nervous systems. When provided with ensembles of afferent sensory information input associated with a specific motor task, e.g., stepping, the spinal cord can "learn" to perform that task even if it is isolated from the supraspinal nervous systems.

The plasticity of the spinal cord led researchers to study the use of physical locomotor training, e.g., treadmill step training with body weight support, to rehabilitate locomotor function after spinal cord injury (SCI). With intensive training, the spinal-cord-injured subject can recover some level of stepping ability. Explorations were made in this thesis to find an optimal training paradigm. Novel assist-as-needed paradigms were developed to allow variability during training since it is an intrinsic feature of normal stepping. Comparative experiments were conducted against fixed-trajectory training. Results demonstrated that variability is an important factor to induce more improvement in step training.

Standing is another important function in one's daily life, though it received less research attention than stepping. A prototype stand platform with 6 degrees of freedom was developed as an experimental tool for stand and postural study. Analogous to step training, we tested the effect of daily training on extensor responses in the hind limbs of complete spinal rats. The results showed no significant effect of the training. This led to the conclusion that without tonic input, the spinal cord has very limited ability to generate enough extensor muscle tone and to respond to postural disturbance. Further studies in standing rehabilitation should combine other

methods to provide tonic inputs to the spinal cord.

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Chapter 1

Introduction

1.1 Motivation

Spinal cord injury (SCI) is a serious clinical problem in modern society. An estimated 253,000 people live with spinal cord injury in the U.S. in June 2006, and an additional 11,000 new cases occur each year [1]. SCI affects primarily young adults, and the major cause of SCI is auto accidents. Since 2000, the average age at injury is 38.0 years. Moreover, the percentage of SCI patients over the age of 60 has increased from 4.7% prior to 1980 to 11.7% since 2000. Spinal cord injury brings many adverse changes to an SCI patient's life. Besides the direct effects of SCI on their body functions, other aspects of their lives, such as employment, mental health, and personal relationships, are also affected. The expenses of health care and living varies according to the severity of injury. For example, the average yearly medical expenses for a paraplegia patient is \$270,913 in the first year of injury and \$27,568 each subsequent year. While for a patient with high tetraplegia, the numbers are \$741,425 and \$132,807, respectively. On all accounts, SCI causes significant increase of life expenses and great impact on the patients' life. Therefore, SCI rehabilitation has every reason to be an important research area.

A complete spinal cord transection will isolate the parts of the body innervated by the spinal cord below the trauma from the upper central nervous system. Clearly, the patients will lose some or all of their volitional control on those body parts depending on the seriousness of the spinal cord injury. A patient with complete spinal

cord transection at the lower thoracic level totally loses control on the bladder function, sexual function, and bowl function as well as the stepping and standing ability. Because of the loss of locomotion ability, SCI patients also endure bone loss [2, 3] muscle atrophy [4] due to chronic unloading of the legs, suffer from muscle spasticity and clonus [5], etc. SCI may also cause chronic pain [6]. All of the above effects have a huge impact on the patient's health and daily life. Intensive research has been conducted on the rehabilitation of different functions after spinal cord injury. Locomotion rehabilitation is of no doubt an important issue. A certain degree of recovery of the locomotion ability will definitely relieve the effort in taking care of the patients and really improve the quality of their daily life as well as their mental well being.

Besides the practical reasons of health care, research on SCI rehabilitation can also bring more insights into the mechanism of the motor control system. The motor control system is believed to have a hierarchical structure where the basic pattern generators lie at the spinal cord level. In complete SCI subjects, the lower spinal cord is isolated from the upper central nervous system and provides us a reduced-order model to study the motor control system.

1.2 Motor System Overview

In order to better understand the subject and the contributions of this thesis, a brief review of the motor control system and the spinal cord is needed.

1.2.1 Segmental Control of Movements

The motor unit is the basic functional unit of the final common path of the motor system. It is defined as an individual motoneuron (MN) and all the muscle fibers it innervates. These motoneurons that directly innervate the muscles via their axons are also called lower motoneurons. The cell bodies of the lower motoneurons are contained in the ventral horn of the spinal cord (figure 1.1, α MN).

There are stretch receptors (proprioceptors) in the muscles, including muscle spindles and Golgi tendon organs (GTOs)(figure 1.1). There are two kinds of afferents

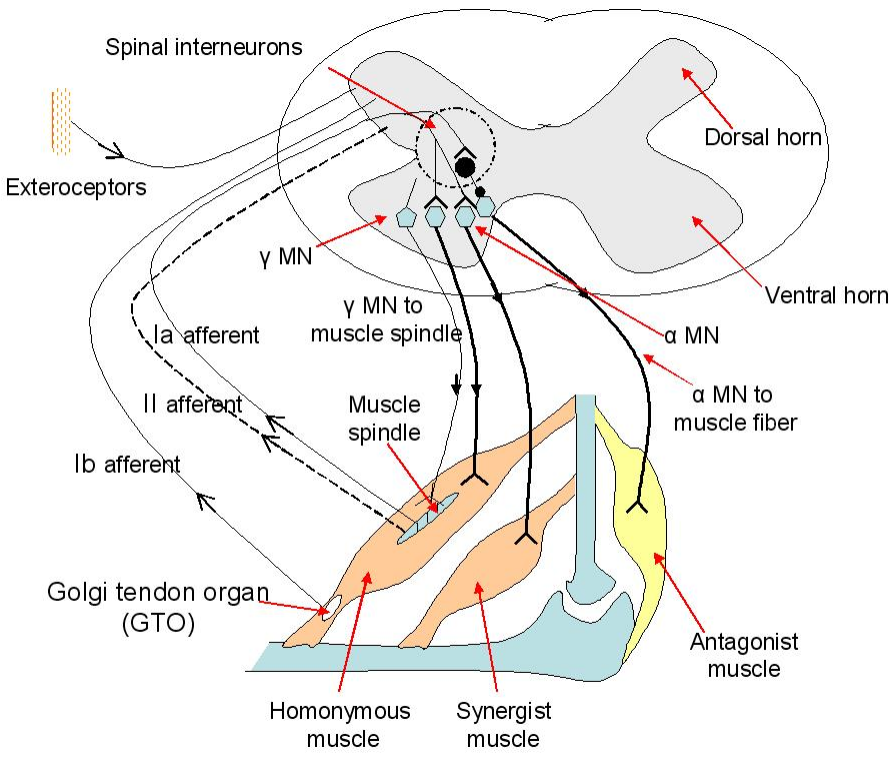

Figure 1.1: Motoneurons and proprioceptive pathways.

from muscle spindles: the primary and secondary muscle afferents. They are embedded within the muscle mass (parallel with the extrafusal muscle fibers), and their sensory components serve as stretch receptors. The primary muscle spindles provide information about both dynamic and static phases of muscle stretch and give rise to group Ia afferents to the dorsal roots of the spinal cord. On the other hand, the firing of the secondary muscle afferents depends on the amount of the stretch and provides primarily static information about the fiber length change. The axons of secondary muscle spindles enter the spinal cord as group II afferents. The sensitivity of the muscle spindles depends on the tone of intrafusal fibers that receive input from γ motoneurons.

The Golgi tendon organs are localized at the junction between the muscle fibers and the tendon. The GTO is in series with the muscle and senses the tension in the muscle, and the information is relayed to the spinal cord via group Ib afferents.

It was first believed that the spinal cord only contained simple reflex pathways. However, from the beginning of the 20th century, evidence accumulated that the

spinal cord is able to generate complex, rhythmic, coordinated behaviors in the absence of supraspinal and peripheral sensory inputs. For example, at the beginning of 20th century, Sir Sherrington found that spinalized dogs and cats were able to generate scratching movements of hind limbs without descending inputs [7, 8]. Furthermore, Brown showed that the spinal cord was still able to generate complex motor patterns after both supraspinal controls and sensory afferents were cut off [9, 10].

Since then, experiments on animals with transected spinal cords brought more and more evidence that the spinal cord itself can generate a variety of complex locomotor patterns, such as walking, scratching, and swimming. For example, local oscillators responsible for crawling and swimming behavior were found in the central nervous system of leeches, as for swimming in lampreys and the beating of crayfish swimmerets [11, 12]. The turtle spinal cord contains central pattern generators that can generate different forms of hindlimb scratching and coordinate the interlimb phase [13, 14]. Numerous studies have been carried out in mammals such as cats and rats, as will be discussed in the next section. Local oscillators are also found in lower animals. For example, in locusts, there are segmental neural circuits that are able to produce rhythmic outputs independently for the activity of single body segments and generate interlimb coordination [15, 16].

All these findings suggest that in the central nervous system, there are local neural networks that are able to generate rhythmic, coordinated locomotor patterns (figure 1.2). The term central pattern generator (CPG) was coined to describe this neural network. A CPG receives modulating inputs from the supraspinal central nervous system, spinal interneurons linking other CPGs as well as the peripheral sensors such as GTO, skin and joints. With a CPG, the motor control system can be organized in a hierarchical way (figure 1.3).

Besides motoneurons and interneurons, the spinal cord also contains descending and ascending pathways that relay information to and from the supraspinal central nervous system (CNS) (figure. 1.3). These pathways are classified by their site of origin: corticospinal or pyramidal tract from the motor cortex, and the extrapyramidal tracts (vestibulospinal tract, rubrospinal tract, tectospinal tract and reticulospinal

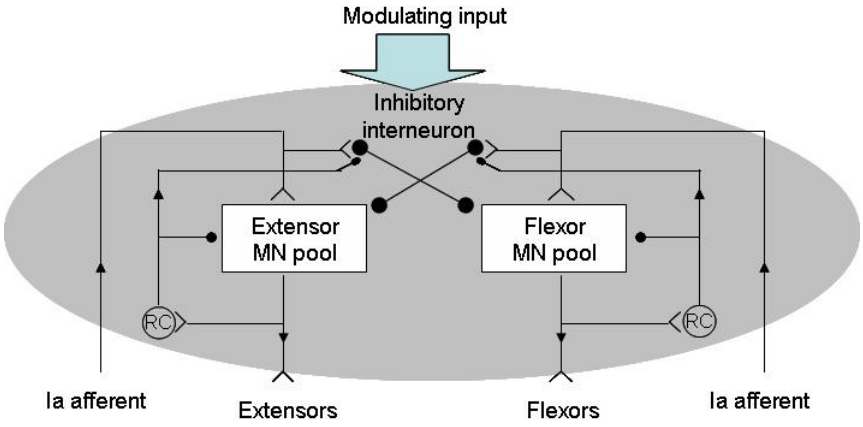

Figure 1.2: A schematic figure of central pattern generator: half center model. Two half centers activate flexors and extensors respectively and mutually inhibit each other.

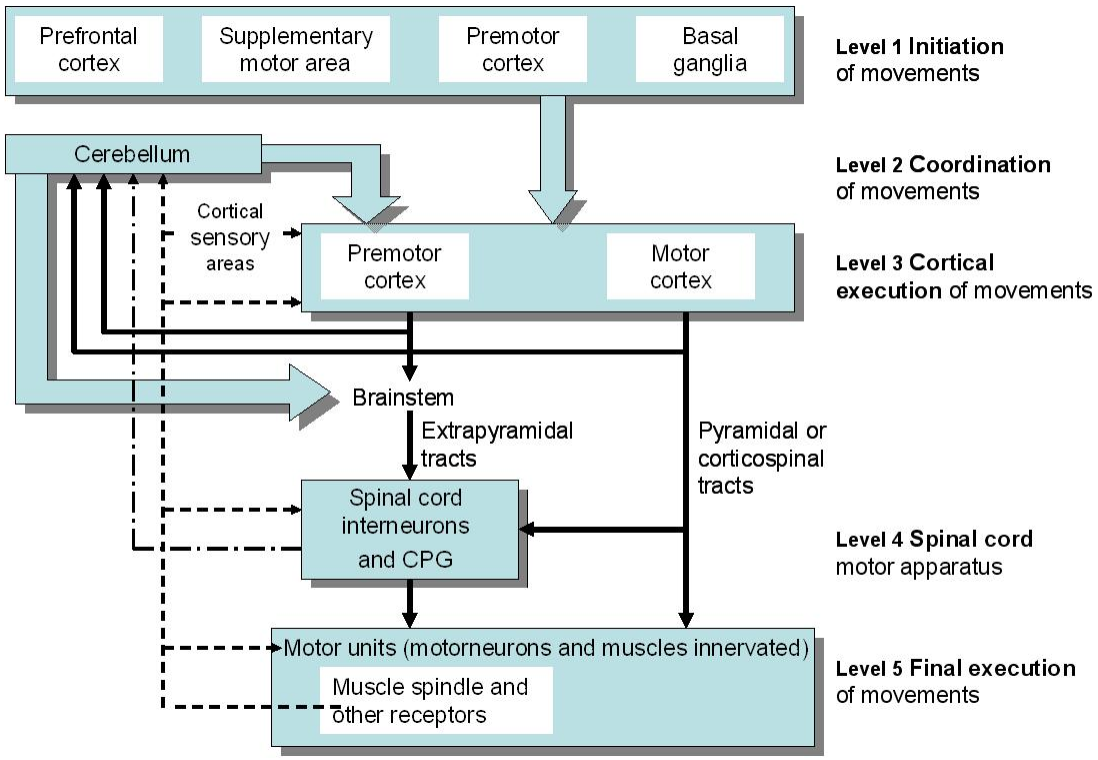

Figure 1.3: The hierarchical structure of the motor system (modified from [17]).

tract), which originate from the subcortical structures. Several different neurotransmitters (e.g., serotonin) that originate from supraspinal nervous system are also transported down the spinal cord via the descending pathways.

The higher nervous centers can send activation commands to CPG networks and modulate the motor patterns. This can be imagined intuitively. For example, when people walk on plain ground, they do not think about all the details of how to lift and drop feet, as well as how to swing their legs. The supraspinal modulation comes into play when there are obstacles or disturbances during the course of walking. Commands from the brain can change the walking pattern to avoid the obstacles. The spinal CPGs are also influenced by sensory inputs. For example, spinal cats walking on treadmill can adjust their gait according to the speed of the treadmill [18].

1.2.2 Spinal Cord Injury (SCI)

Spinal cord injury is a trauma to the spinal cord. The motoneurons as well as the descending and ascending pathways in the injured segment are damaged. Depending on the position and the characteristic of the trauma, different functional deficits are generated.

Clearly, a complete spinal cord injury isolates the lower spinal cord (spinal cord below the level of trauma) from descending inputs. For example, after complete SCI, the subject will lose voluntary control of the body parts innervated by the spinal cord below the transection point. In addition to the loss of voluntary control, the motoneurons and interneurons below the injury point will lose some excitatory and inhibitory inputs from the descending pathways. Some neurotransmitters or neuromodulators (chemicals that relay information between neurons) have supraspinal origins and are relayed to the spinal cord via the descending pathways. After spinal cord injury, the supply of these neurotransmitters is diminished. This causes change in the biochemical environment in the spinal cord. Due to the above reasons, the motor behavior that is generated via the lower spinal cord is changed after SCI.

The signs and symptoms after SCI evolve over time. Immediately after the com-

plete SCI, there is a period of spinal shock. During the spinal shock period, automatic reflexes in the segments at and below the level of injury are suppressed (areflexia or hyporeflexia) and somatic sensation is lost. This condition is called flaccid paralysis. The spinal shock could last one or several weeks after SCI.

After spinal shock, there is a gradual return of segmental reflexes in the lower spinal cord. At the level of injury, the segmental reflexes never return because the circuitry at that level is destroyed. Over the course of several weeks, the reflexes in the lower spinal cord become hyperactive (hyperreflexia).

Over several months, hyperreflexia continues to develop to the point of spasm of large amplitude and becomes disturbing to the patient. Muscle clonus (alternating flexion and contraction) is another symptom. This condition is termed spastic paralysis.

Also the muscles affected by the SCI undergo atrophy. Especially, in those muscles innervated by the injured segments, there are complex changes leading to alterations in membrane properties, muscle atrophy, and changes in ion channels and neurotransmitter receptors.

Research in SCI rehabilitation can bring us insights into the mechanisms of CPGs and motor control organization, and therefore help us to better understand the motor control system.

1.3 Studies in SCI Rehabilitation

For over 30 years, SCI rehabilitation has been an active research field and will still be in the future [19, 20, 21]. Researchers have been studying the potential of harnessing the spinal neural circuits which remain intact below the trauma position [22]. Different methods have been used to enhance locomotor recovery. To date, There have been 3 major directions in rehabilitation research and practice. First, intensive rehabilitative training (e.g., step training on a treadmill with body weight support) can promote locomotion ability after SCI [23]. Extensive study has been conducted in cats, primates (human and monkeys) as well as rodents (rats and mice). Step training

can affect the neural pathways [24] as well as the biochemical environment in the spinal cord [25, 26]. Second, neural regeneration methods are also being studied to reconnect the injured spinal axons and repair the neural circuits [27, 28, 29, 30]. And third, Functional Neuromuscular Stimulation (FNS) or Functional Electrical Stimulation (FES) [31], which can generate functional movements by electrically stimulating the neural or muscular tissue, are also being studied to restore or improve locomotion ability [32, 33, 34, 35] and commercial FES products have been developed [36].

The focus of the work in this thesis is the effect of physical training on SCI rehabilitation, so we will focus the discussion more on related work in rehabilitative training from now on.

One of the most important findings in the research of spinal cord CPGs is that the spinal cord circuitry is responsive to activity-dependent (or task-dependent) interactions, which enables the harness of the CPGs to regain locomotor function (at least to a certain degree) after spinal cord injury.

The plasticity of the spinal cord arises from several aspects. The neural circuitry can be reorganized over time in healthy subjects. For example, people can always learn to perform new motor skills. After SCI, the neural pathways and interneuronal circuits will undergo significant reorganization due to the loss of descending supraspinal inputs and the chronic unloading of the legs [37, 38]. And the biochemical environment will also undergo changes since some of the neurotransmitters originate from supraspinal sites and the injury interferes or even precludes their transport down the spinal cord [38].

Plasticity is also manifested by the spinal cord when it learns to perform a certain motor task (e.g., stepping) when provided with the appropriate sensory information associated with that motor task. Also the biochemical environment can be modulated by training [38]. For example, locomotion and postural training can modulate the inhibitory potential of the spinal cord of the adult spinal cat [25, 26].

The activity-dependent plasticity of the spinal cord in either healthy or diseased subjects has been reviewed by many researchers [23, 37, 39, 40, 41]. With repetitive training, the lower spinal cord can learn to perform the trained task [38, 42].

The cat model has been used to study fictive locomotion. In decerebrate cats, stumbling corrective reactions in response to stimulation of the superficial peroneal (SP) nerve were observed [43]. The reaction results from different intracellular pathways including the interneurons in lumbar spinal cord involved in the generation of fictive locomotion [44].

In cats spinalized at young age, full weight-bearing locomotion can be recovered although the locomotion pattern is quite different from that of a normal kitten [45, 46]. But in cats spinalized as adults, the locomotor ability has significant deficits without training, including inability to bear the weight of hindquarters, high variability in step cycle, and loss of coordination between hind limbs [47].

Recent evidence shows that with intensive step training on a treadmill, adult spinal cats (spinalized at lower thoracic level) can regain a much greater ability in regulating hindlimb locomotion than those allowed to recover spontaneously [48, 49]. The general stepping pattern was remarkably similar to that of normal cats though several deficiencies were observed [47, 50, 51]. These results provide evidence for spinal CPG in cats and the benefits of rehabilitative training.

The locomotion activity is also affected by peripheral inputs. For example, in the absence of cutaneous inputs from the hindpaw, there are long-term changes in locomotion pattern in intact cats, especially in demanding conditions such as ladder walking (requiring precise foot positioning) [52]. Also in spinal cats, cutaneous inputs from the hindpaw plays a critical role in locomotion [53]. Complete cutaneous denervation in spinal cats caused failure in plantar foot placement and full weight-bearing stepping. Partially denervated cats can readily recover those abilities, suggesting that the spinal cord has adaptive capacity.

Besides physical training, pharmacological intervention also has an important role in the generation of locomotion activity. Clonidine (a noradrenergic alpha-2 agonist) can initiate locomotion early after spinalization in cats. Combining clonidine administration with intense treadmill training was shown to be able to enhance the locomotion recovery [54]. Noradrenergic and serotoninergic drugs were also shown to help in stepping rehabilitation [55, 56].

Rehabilitation studies have also been conducted in rats and mice [57, 58, 59, 60]. In these studies, spinal rats and mice regained stepping ability to some degree after intensive step training combined with pharmacology intervention. Also the efficacy of robotic devices in rehabilitative training was demonstrated [61].

In other words, Step training can induce plastic changes in the neural pathways [62] as well as the biochemical environment [25, 26] in the spinal cord, which provides the potential to harness the spinal cord circuitry to restore locomotion ability after an SCI.

These observations are consistent with the existence of a Central Pattern Generator (CPG), i.e., a neural circuit in the spinal cord that can generate the primitive stepping patterns without the input from the higher central nervous system and peripheral sensory information [63].

There is no conclusive evidence that CPGs also exist in the human spinal cord. But there is evidence that humans with complete or incomplete spinal cord injury can regain a certain amount of locomotion ability after intensive locomotor training with partial body weight support on a treadmill [40]. Rehabilitative training with partial body weight support has been applied to patients with SCI (incomplete or complete), stroke, Parkinson's disease, etc. [64, 65, 66, 67, 68, 69, 70]. Functional Electrical Stimulation (FES) was also combined with step training for locomotion rehabilitation [71, 72]. It seems that the human lumbosacral spinal cord has the ability to interpret the afferent information such as loading and velocity during stepping [73, 74, 75]. Some patients regained limited ability of walking and standing after training [76]. Even if the patients do not learn to walk, the training seems to have secondary benefits, such as more muscle activities and circulation, fewer spastic movements, fewer bedsores and other infections [4, 77, 78].

While step training has traditionally been conducted manually, robotic technology has recently been applied in rehabilitation research [57, 60, 79, 80]. In manual training, the subject is put on a treadmill with appropriate body weight support so that the subject can maintain an upright posture. The legs are guided manually by the therapists or trainers along a trajectory close to normal stepping so that the loading

and unloading information and proprioceptive information of walking are delivered to the spinal cord. This is a physically demanding procedure and a strenuous task for the therapists. Also the consistency of the training cannot be guaranteed due to various factors such as fatigue of the therapists and the training effect depends on the experience of the therapists. Robotic training follows the same principle except that the guidance of the limb movement is provided by an actuated orthosis or robotic arms. For example, one commercially available robotic device, the LOKOMAT [81], was developed at the University Hospital Balgrist in Zurich. Besides alleviation of human efforts, robotic training has several advantages over manual training: (1), consistency can be guaranteed during the training process; (2), training are parameterized and controlled by computer so that it's easier to study different training strategies; (3), training strategy can be easily adapted for different subjects; and (4), robotic device can provide good quantitative information on the performance of the trainees.

It follows naturally from the above discussion that SCI rehabilitation is an important and deserving research topic. Moreover, ample prior research has suggested the use of physical therapy, possibly coupled with drug therapy, as an important component of locomotion recovery in SCI patients. Although robotic technology has played an important role in these studies, further efforts are needed to better utilize the capacity of robotic devices. So the first part of the thesis explores novel robotic step training paradigms. On the other hand, standing rehabilitation is less studied than stepping even though standing is a very important function in one's daily life. In this thesis, a pilot study on how to facilitate standing recovery is conducted.

1.4 Outline of the Thesis

This thesis includes studies on both stepping and standing rehabilitation in animal models of mice and rats, in which robotic training was employed. It was shown that robotic technology is an effective tool in rehabilitation training. And the results provided further insights into the mechanism of locomotion control.

The thesis will be organized as follows. In chapter 2, a stepping rehabilitation study of a mouse model was conducted. Some exploration on optimal robotic step training was made. Results showed that allowing variability in the training paradigm employed the intrinsic automaticity of the spinal cord circuitry and had a better training effect than the original rigid training paradigm along a fixed trajectory.

Inspired by the efficacy of robotic device in repetitive step training and the plasticity of spinal cord in stepping rehabilitation, a pilot exploration in standing rehabilitation using robotic device was made in this thesis. In chapter 3, a prototype device was developed for the purpose of stand training and posture control. The device is a 6-DOF parallel platform combined with a body weight support system. Using this device, the effect of stand training using repetitive perturbation to the hind limbs was studied in rats (chapter 4).

Finally in chapter 5, concluding remarks on the thesis are included and possible future directions are discussed.

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Chapter 2

Assist-as-Needed Step Training: The Effect of Variability

The work in this chapter was done in collaboration with Dr. Lance Cai and the results were published in J. Neurosci. (2006) 26(41):10564-10568

2.1 Introduction

As discussed in the first chapter, the spinal cord circuitry has a great degree of plasticity so that when provided with appropriate afferent information of a motor task, e.g., stepping, it may learn to perform that task without the input from the upper central nervous system [1, 2].

Step training has been conducted manually or robotically [3, 4]. The basic idea of the training is to guide the legs of a spinal subject along the normal stepping trajectory [5]. Since the subject cannot support its body weight totally by itself, partial body weight support is provided to help the subject maintain an appropriate posture on a moving treadmill. In this way, the afferent neural inputs during normal stepping, such as ground contact, pressure, and joint angles are generated and delivered to the spinal cord.

Manual training is a strenuous work for the physical therapists and trainers. Also the training highly depends on the experience of the trainer. Robotic devices have been more widely used recently. Robotic devices are controlled by computer, and therefore can save lots of human effort. Robotic training has been shown to be

successful in rats, mice, and human beings [4, 6, 7, 8].

The training strategy usually used in robotic training involves movement of the trainees' limbs along a specific trajectory via tight position control of the robot linkages, e.g., the LOKOMAT robot [8]. The robotic device simply guides the legs along a fixed trajectory, i.e., there is no variability allowed during the robotic training [6, 7]. In this sense it is different from manual training. Manual training usually allows a certain amount of freedom to the subjects' limb movements based on their performance. For example, if the subject is doing well, i.e. the stepping trajectory is close to the preferred trajectory, the guidance needed can be less rigid. Otherwise, more effort will be needed to guide the legs to the training trajectory. In this way, a compliant interaction between the trainer and trainee is allowed, and the automaticity of the spinal cord circuitry is better employed. In fact, as a fundamental feature of natural locomotion, there is variation in the limb's movement kinematics and motor activation patterns from cycle to cycle during repetitive movements such as stepping [9]. A robotic device driven by a fixed trajectory in fact could limit the degrees of freedom of the leg's motion compared to natural motor activity patterns [10]. As a consequence, this could reduce the activity of the neural circuits [11] and might also induce "learned helplessness" in the lower spinal cord, i.e., habituation to repetitive activation of the same sensory pathways during a training session [12, 13].

This chapter tests the hypothesis that allowing variation in the pattern of limb movements during training could provide more natural sensory information to the spinal circuits and thus lead to better training effect. A new robotic training strategy, assist-as-needed (AAN) training was implemented on a robot scaled for mice step training (figure 2.1). Two heuristic control algorithms were tested in adult spinal mice. They both allow a certain amount of freedom along the training trajectory. One of the algorithms integrates phasic coordination between left and right legs while the other has looser restriction on leg coordination.

Comparative experiment between the fixed-trajectory training paradigm and the two AAN training paradigms was conducted in 27 mice, which were randomly divided into 3 equal groups. Results showed that AAN training with leg coordination resulted

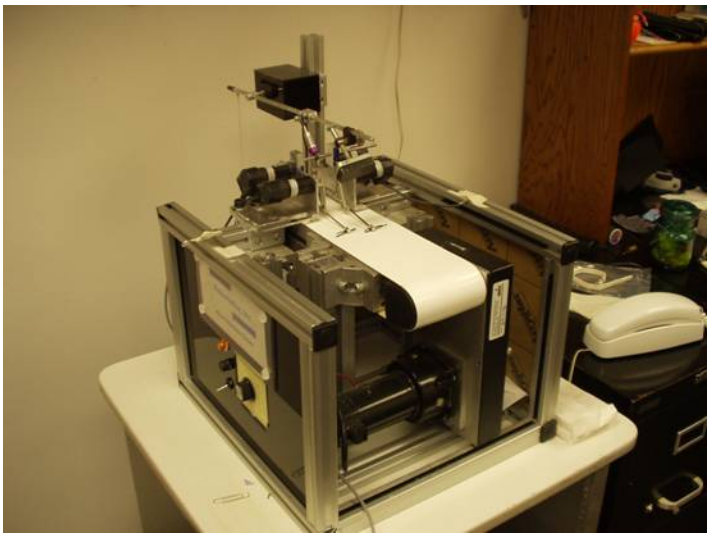

Figure 2.1: The robot used for step training in mice.

in more improvement to the stepping performance of the trained mice, especially in terms of the number of steps and stepping rhythmicity.

2.2 The Step-Training Robot

A four-axis robotic system (figure 2.1) was used for both active training and data acquisition of mouse limb movements. figure 2.2 is a schematic view of the robot.

The system enables independent, two-dimensional tracking and control of each ankle in the sagittal plane as the mouse steps on a moving treadmill. It consists of four major components: (1) a pair of robotic arms, (2) a motion controller board, (3) a treadmill, and (4) a body-weight support device. The robotic arms, the primary components of the robot, serve as the interface between the electronics and the mouse. Each robotic arm is composed of a five-bar leg-guidance linkage, a pair of motors that drive the linkage (2342-006CR; Micromo Electronics, Clearwater, FL), and a pair of optical encoders that record the rotational position of the motors (HEDM-5500; Agilent, Palo Alto, CA; Micromo Electronics). Forward kinematic equations are used to derive ankle position from the motor angles. The leg-guidance linkage is sized to enable motion tracking and control within a 3.5×3.5 cm workspace, which is sufficient to accommodate all step trajectories associated with mouse treadmill loco-

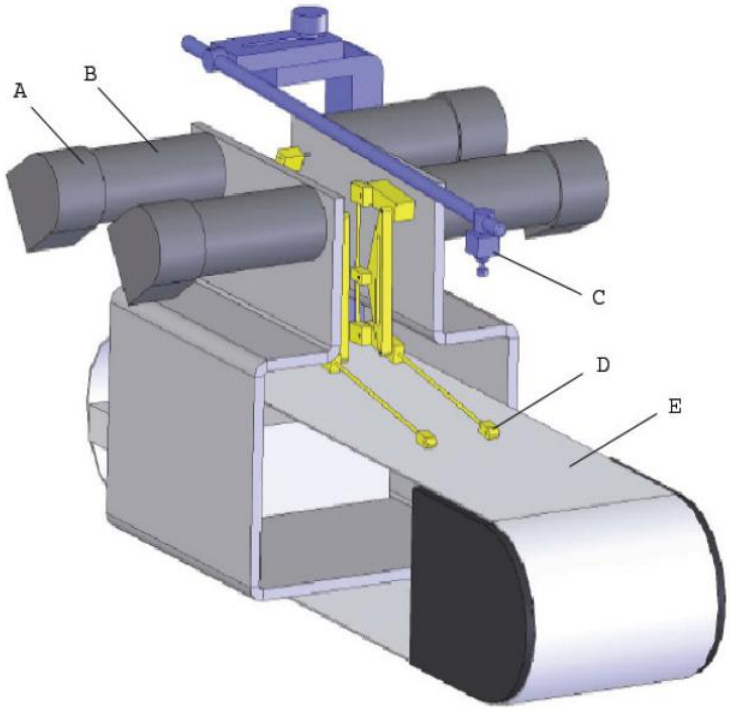

Figure 2.2: Schematic of the step training system. Important components are labelled: (A) Optical encoder; (B) Motor; (C) Weight support; (D) Manipulators; and E) Motorized treadmill.

motion. The robotic system was used in two modes. In its active training mode, the robotic arms can drive the hind limbs through any predetermined pattern within the workspace. In its passive recording mode, the linkages move freely in the workspace, allowing the computer to record independent ankle movements generated entirely by the mouse. To minimize encumbrance on the mouse hind limbs, precision bearings and motors with low internal friction are used at all revolute joints. The frictional resistance force at the end effector is estimated to be 0.032 N. The mass inertia of the robotic arm linkage, including its actuators, is estimated to be $\sim$ 0.4 g in its reference configuration, the orientation of the robotic arms in which the stepping workspace is initialized. Mass inertia remains within the same order of magnitude of this value for all orientations within the feasible stepping workspace of the mouse. These are practically negligible values. The robotic arms do not critically hinder stepping (see videos at http://robotics.caltech.edu/jneurosci). All mice used in the study were tested under the same conditions on the robot. A 4-inch-wide industrial conveyor (GUF-P 2000; MK Automation, Bloomfield, CT) was modified for use as a treadmill. The stock belt was replaced with a slightly tacky treadmill belt that provided a slip-free stepping surface without irritating the skin on the paws, an injury commonly observed with other belt materials. Mice are placed into the mouse stepper using a cone-shaped cloth harness that is magnetically secured to the weight-support system. The hindlimbs are connected to the robotic arms using a drawstring loop attachment. Both ends of a rounded rubber string are fed through an eyelet in the linkage end effector, forming a loop through which the hindpaw is placed. The diameter of the eyelet is sufficient to pass both ends of the string but large enough to resist axial slipping when the string is drawn tight. The thickness of the rubber prevents the ankle from coming into direct contact with the metal linkage, and its elasticity allows an appropriate amount of rotation and lateral motion while the ankle is guided through sagittal trajectories. The robotic system enhanced both locomotor training and recording, providing several benefits: (1) it enabled precise control of hindlimb movements, (2) it ensured consistent application of a training protocol between mice and across training sessions, (3) it provided a quantitative record of the

training history of each mouse, (4) it granted immediate access to the data, and (5) it facilitated application of quantitative analysis techniques.

During training the speed of the treadmill was adjusted to match the speed of the robotic device. Typically, this speed was 3 cm/s [7].

So that the AAN algorithms could be implemented, precise control of the robotic linkages was required. Therefore, a four-axis dedicated controller board (DMC-2240, Galil Motion Control, Rocklin, CA) was used. The control output was sent to the motors via an interconnection module with an integrated amplifier (ICM/AMP 1900; Galil Motion Control). The control algorithms were written with LabVIEW (National Instruments, Austin, TX), and their output commands were sent to the controller board via an Ethernet connection. The feedback commands were updated at 200 Hz. A lower level PID controller is integrated with the controller board. The controller gains can be manually set to obtain optimal performance.

2.3 The Algorithms

New training paradigms with adaptivity have been developed recently. For example, adaptive control algorithms were designed for LOKOMAT [14] based on minimizing the interaction forces between the patient and the orthosis. In our mice stepper, it is not realistic to mount force sensors on the robotic arms due to the device's size. Additionally, it is not necessarily true that algorithms based on force feedback provide the best avenue to optimize post-SCI spinal cord learning. Two open-loop algorithms were designed to allow variation during the step training. The key hypothesis that these algorithms test is that natural limb motion variability during the training leads to improved spinal learning. The common idea between the two algorithms is to set a boundary along the training trajectory. When the animal is moving within the bound, restrictions are imposed on the animal by the robot arms, but with as little assistance as possible, i.e., just enough to move the legs along the direction of the trajectory. When the animal moves out of the bound, the robot will guide it back to the bound so that it will not move too far away from the training trajectory. In

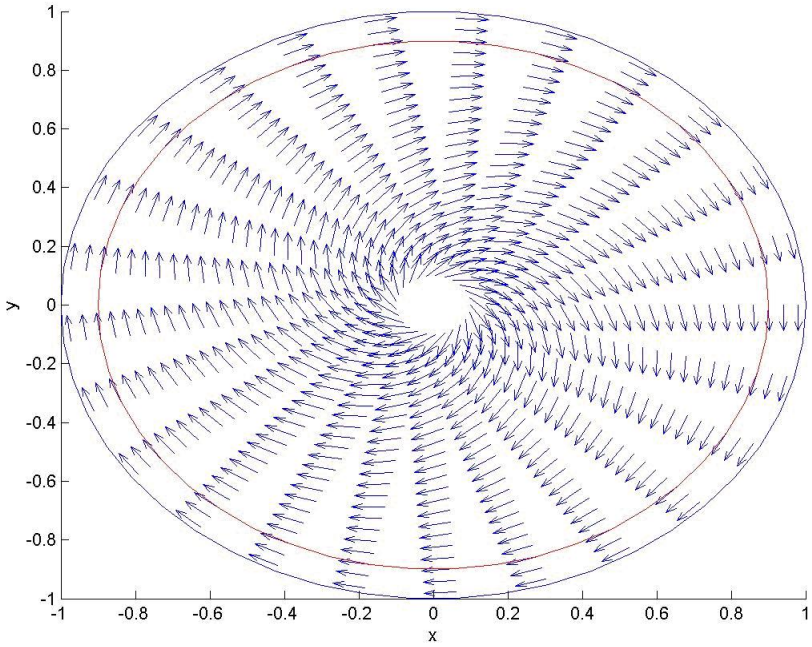

Figure 2.3: A banded velocity field inside a unit circle.

this section, the implementation of the two algorithms will be presented. The first one is called band AAN training. A converging velocity field is used to control the movement of the robot linkages. The velocity field is generated for the end effector of the robotic arm. Then the velocity can be transformed into motor velocity by the inverse Jacobian of the robotic linkages.

2.3.1 Band Training Algorithm

The concept of the final design of the velocity field that defines the control algorithm starts with a circular converging velocity field.

In figure 2.1, a banded velocity field is depicted. The inside circle defines a band along the unit circle. Within this band, the velocity field moves tangentially along the circle in the clockwise direction. Outside the band, the velocity has two components, one tangential and one normal. The tangential component keeps the robot moving along the desired direction while the normal velocity will lead the end effector back to the band. The lower level control is implemented through the PID controller

integrated in the controller board. Thus, inside the band, the subject's limbs can move freely in the normal direction, which gives the trainee some freedom to allow spinal automaticity to function. Outside the band, the trainee will be guided back in a more gentle way than in the fixed trajectory training, in which precise position control is used to lead the trainee along the training trajectory. In fact, the rigidity difference of the robotic arm between the two cases can be obviously felt when applying a disturbance to the linkages by hand when the linkages are moving.

The velocity field in figure 2.3 is defined as follows:

$$\vec{v}_r(r,\alpha) = (1-\delta-r)(\vec{i}\cos\alpha + \vec{j}\sin\alpha), \qquad r \le 1-\delta, \tag{2.1a}$$

$$\vec{v}_r(r,\alpha) = 0, \qquad r > 1-\delta, \tag{2.1b}$$

$$\vec{v}_\alpha(r,\alpha) = k(-\vec{i}\sin\alpha + \vec{j}\cos\alpha). \tag{2.1c}$$

Here the subscript r stands for radial component, α stands for the angular component and k is a constant. $\vec{i}$ and $\vec{j}$ are the unit vectors along the $x-$ and $y-$ axis, respectively. Distance $(0 < \delta < 1)$ defines a band along the unit circle. If the point is within the distance to the unit circle, the velocity field just follows the trajectory. Outside the band, the velocity field tends to move toward the band.

Once we have this original velocity field, numerical conformal mapping is used to map it into the velocity field along the actual training trajectory. First, a conformal mapping that maps the unit disk (area inside the unit circle) into the inside of the actual trajectory was determined using the unit circle and the training trajectory. Using this mapping, the clockwise velocity field was mapped into a clockwise velocity field inside the training trajectory and the convergent property was inherently conserved by conformal mapping. To get the velocity field outside the trajectory, simply reverse the rotating direction of the unit circle velocity field and map it into the outside area of the trajectory. The mapping is again determined by the unit circle and the actual trajectory while the target area is different. Combining these two velocity fields resulted in the overall velocity field, we generated the full velocity field used for step training (figure 2.4).

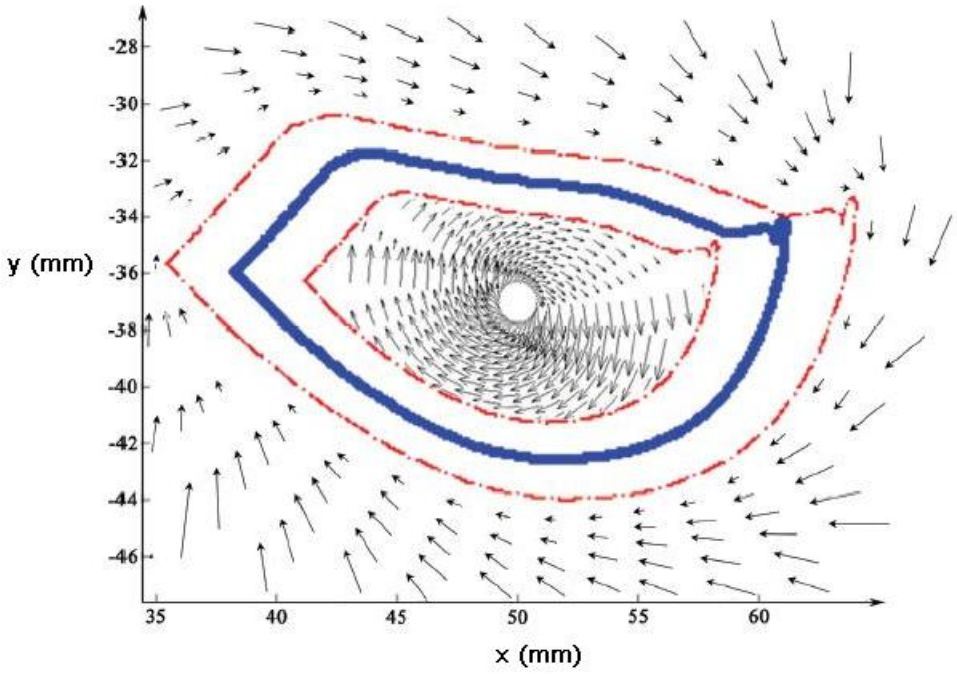

Figure 2.4: The banded velocity field used in training. The solid thick line shows the desired training trajectory of the animal's ankle position in the sagittal plane. The dotted thin lines represent the boundaries within which soft control is applied to the limbs. The arrows outside the boundaries correspond to the convergent velocity fields that drive the legs to the band region.

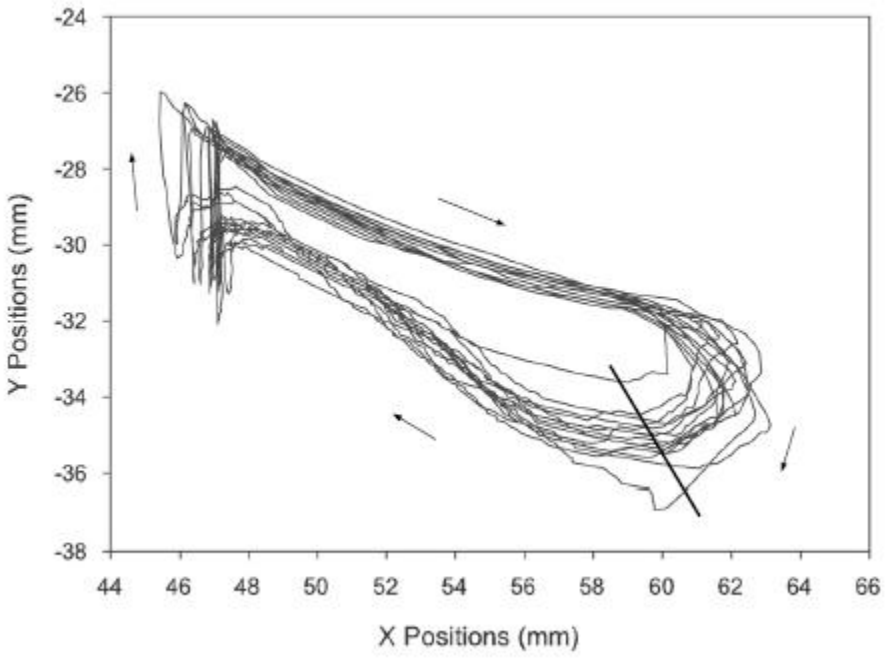

Figure 2.5: The stepping trajectories of the ankle of a neonatally spinal cord transected mouse at approximately three months of age. The diagonal line through the trajectories shows where the most deviation (~ 4 mm) occurs. The arrows represent direction of travel.

The original band width was adjusted such that after conformal mapping, the actual band width was consistent with the statistic variation of the stepping trajectory of neonatal transected mice. The amplitude of the tangential velocity along the training trajectory was adjusted automatically by the numerical conformal mapping since the data points of the training trajectory were recorded at equal time intervals. The numerical conformal mapping used here was implemented via the Zipper program developed by Professor Donald E. Marshall of University of Washington (http://www.math.washington.edu/ marshall/zipper.html).

In training, the same velocity field is used for both sides. Phasic coordination is loose in this case since only the initial positions of two hind feet were placed at the beginning of the training session and the positions are not firmly controlled during the training period.

2.3.2 Window Training Algorithm

The other AAN training algorithm is different in the sense that it integrated the phasic coordination information between left and right legs. The idea is to put a moving window along the training trajectory (so this algorithm is called the "window algorithm"). The control velocity field again is defined based on the position of the end effector. In the former band algorithm, the velocity field is a function of the absolute position of the end effector. While in the new method, it is a function of the end effector position relative to a moving window that moves along the training trajectory with phasic coordination between two sides of the animal's body. Inside the window, the velocity field is tangential to the local trajectory and the amplitude is adjusted according to velocity calculated from the original trajectory data. While outside the window, another velocity field is superposed to the tangential field. This other velocity field moves the limbs back toward the inside of the moving window. For simplicity, a circular window was used in the experiment. Again the radius of the window is decided by the variation of the neonatal stepping trajectories, which is 4 mm in the current experiment. In this case, the second velocity field is a radial field pointing towards the center of the window, and the amplitude is determined by:

$$v = k(d - r), \tag{2.2}$$

where d is the distance between the current location of the ankle and the center of the moving window, r is the radius of the window and k is a constant.

2.4 Experimental Study

2.4.1 Animals and Animal Care

Adult female Swiss-Webster mice (mean body weight of 25.3 ± 1.3 g on the day of spinal cord transection) obtained from Charles River Laboratories (Wilmington, MA) were used. The mice were housed individually, had access to food and water

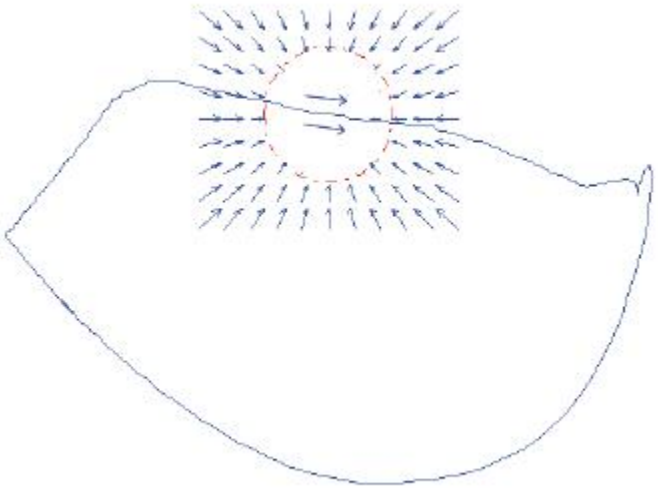

Figure 2.6: The moving window AAN strategy. The solid line represents the desired training trajectory of the animal's ankle position in the sagittal plane and the moving window is outlined by the dotted circle within which soft control is applied to the limbs. The arrows outside the circle correspond to the superposed radial velocity fields.

ad libitum, and were kept on a 12 h light/dark cycle for the duration of the study. All animal procedures used in these studies were conducted in accordance with the Animal Care Guidelines of the American Physiological Society and were reviewed and approved by the Animal Research Committee at the University of California, Los Angeles, where these experiments took place.

2.4.2 Surgical Procedures and Postsurgical Care

Surgeries were performed at approximately postnatal day 60 (P60). The surgical procedure used is the same as the one reported in [7]. Briefly, the mice were maintained in a deep anesthetic state throughout the surgery with isoflurane gas ($2\% - 5\%$ isoflurane mixed with 0.4% O_2 via face mask). All procedures were performed under aseptic conditions. The mice were placed on a heating pad to maintain body temperature. A skin incision was made along the dorsal midline from T6 to T9 to expose the musculature overlying the vertebrae. The paravertebral musculature was

retracted to expose the vertebral column, and a laminectomy was performed from T7 to T8. Gel foam then was inserted into the gap to ensure complete separation of the proximal and distal stumps [15]. The musculature and fascia were repositioned, and the wound was closed using 5.0 Dexon internal and 5.0 Ethilon external sutures [7]. The mice were placed in an incubator maintained at 29 ± 1 ° C and observed until fully recovered from anesthesia after surgery. The mice were returned to their cages and given Baytril (40 mg/g body weight), a broad spectrum antibiotic, via drinking water for 14 days. Post surgical care and maintenance procedures were similar to those described previously for SCI rats and cats [16, 17]). The bladders of the spinal mice were expressed manually twice daily to minimize the risk of bladder infection and related complications. After bladder expression the hindlimbs of the mice were stretched lightly once through a full range of motion to help sustain joint mobility. Food rewards were given to stimulate positive interaction between the mouse and handler.

2.4.3 Euthanization

At the conclusion of each study, the mice were anesthetized deeply with sodium pentobarbital (50 mg/kg) and transcardially perfused with 1 mL/g body weight of 0.1 M phosphate buffer, pH=7.4, followed by 2 mL/g body weight of 4% paraformaldehyde for 12 min. The spinal cords then were removed, post-fixed in 4% paraformaldehyde for 5 hours, and cryoprotected by incubating in a 30% sucrose solution in 1X phosphate buffered solution overnight to ensure adequate absorption. The cords were then frozen on dry ice, and stored at -80 ° C.

2.4.4 Quipazine Administration

Serotonin agonists are known to modulate or induce locomotion in rats [18] and mice [19]. In this study quipazine, a broad spectrum serotonin agonist, was used to facilitate stepping in all mice. Based on our own dose-response tests, as well as dose-response studies reported for rats [7, 20], a dose of 0.5 mg/kg body weight was

administered intraperitoneally 10 min before each training or testing session.

2.4.5 Step Training

Twenty seven mice were randomly and equally divided into 3 groups (9 in each group). Two of the groups received the AAN band and window training methods described before and the other received fixed trajectory training so that the effect of variability can be studied.

To be clear, in the case of fixed trajectory training, the end effector of the robot arm is controlled by a Proportional-Integral-Derivative (PID) controller integrated with the motion control board to track a desired stepping trajectory, which was obtained from neonatally transected spinal mice (they can recover functional stepping ability close to normal level).

All three groups were trained for 10 minutes each day, five days a week, and for 6 weeks. Before training, Quipazine was given to each mouse through IP injection (injection into the peritoneal cavity). Training began 10 minutes after the injection, allowing time for the drug to take effect.

2.4.6 Data Analysis and Evaluation Methods

Testing was performed on the fifth day of each training week. The mice were given a 2 min "warm-up" period before each testing session, using the same training algorithm associated with that particular group. The mouse stepper was used in a passive recording mode to track the ankle position of each leg for 2 min at a treadmill speed of 3 cm/s. Position data were recorded at 200 Hz, using a custom acquisition program written in LabVIEW (National Instruments). In addition to the robot data, video footage of both the left and right sides of each mouse was captured during testing, and a log of qualitative observations was maintained. Using these data, we assessed the quality of stepping in terms of (1) the number of steps performed; (2) the periodicity of the steps, i.e., the ability to maintain a regular stepping frequency; and (3) the shape regularity of the stepping patterns. The following analyses were

used for these assessments of stepping ability.

Number of Steps

Video footage and plots of ankle position data were used to identify steps and the number of steps performed by each mouse during the 2 min testing period. Steps were identified on the basis of predetermined criteria for step length (minimum 5 mm), height (minimum 5 mm), duration (minimum 0.5 s/maximum 1.5), and degree of interlimb coordination [7]. On each testing day the 12 s stepping interval containing the most steps was recorded for subsequent analysis.

Step Periodicity

Fast Fourier transform (FFT) analysis was applied to the horizontal component of the stepping trajectories to quantify step periodicity [7]. Mice that stepped rhythmically exhibited a sharp and distinct fundamental peak in the FFT spectrum of their ankle trajectories. The location of the predominant peak corresponds to the fundamental stepping frequency. Conversely, mice with poor stepping periodicity either exhibited a very broad fundamental peak in the FFT spectrum or, in extreme cases, failed to demonstrate a fundamental peak. To quantify these observations, we measured the full width at half maximum (FWHM) of the fundamental peak. A low FWHM value corresponds to temporally consistent, rhythmic stepping, whereas a high value typically indicates erratic stepping, consistent with stumbling and foot dragging.

Spatial Consistency

To measure consistency of stepping systematically, principal components analysis (PCA) [7, 21] was used. PCA is a multivariate analysis technique that picks out patterns in a data set and reduces the dimensionality of the data without significant loss of information. Given a series of step trajectories, PCA extracts the fundamental trajectory as the first principal component. The PCA score reported here is the percentage of the total variance that is captured by the first principal component. Hence, the higher the PCA score, the more consistent the stepping.

The raw stepping data were preprocessed for PCA. First, the successful step cycles from the selected best $12s$ intervals of stepping were isolated and separated into their

x and y components. Each step component was resampled to a consistent number of data points per step, thus removing the temporal information. The data were then arranged into two $m \times n$ matrices, with each column containing the data for a single step and each row containing the interpolated position values at each time step. A custom program written in Matlab (MathWorks, Natick, MA) development environment was used to identify the principal components of each dataset and to calculate the PCA score. A minimum of three successful steps was required to conduct a statistically significant PCA. A minimum PCA percentage of 35% was assigned to all mice that could not step on the test day, because this was the lowest PCA score that was encountered [7].

2.5 Results

All groups showed improvement over the 6-week training period based on the average number of steps taken in the best 12-second interval during each testing session (figure 2.7). The mice in the Window group, however, had a faster rate of recovery than in the other two groups. The average number of steps taken by the Window group was higher than in the Fixed group from weeks 1 to 3 and higher than in the Band group at weeks 1 and 3. There were no significant differences between the Band and Fixed groups at any time point, and the average number of steps was similar in the three groups after 6 weeks of training.

Inverse FWHM scores for the mice in the Window group were significantly higher than in the Band and Fixed groups after 4, 5, and 6 weeks of training (figure 2.8). There were no significant differences between the Band and Fixed groups at any time point. The maximum level of step rhythmicity was achieved after 6 weeks of training: Window group (8.3) was higher than the Band (6.4) and Fixed (6.3) groups.

All three groups showed progressive improvement in step shape consistency, based on PCA analysis, throughout the first 5 weeks of training and then a slight decrease at week 6 (figure 2.9). There were no significant differences among the three groups at any time point.

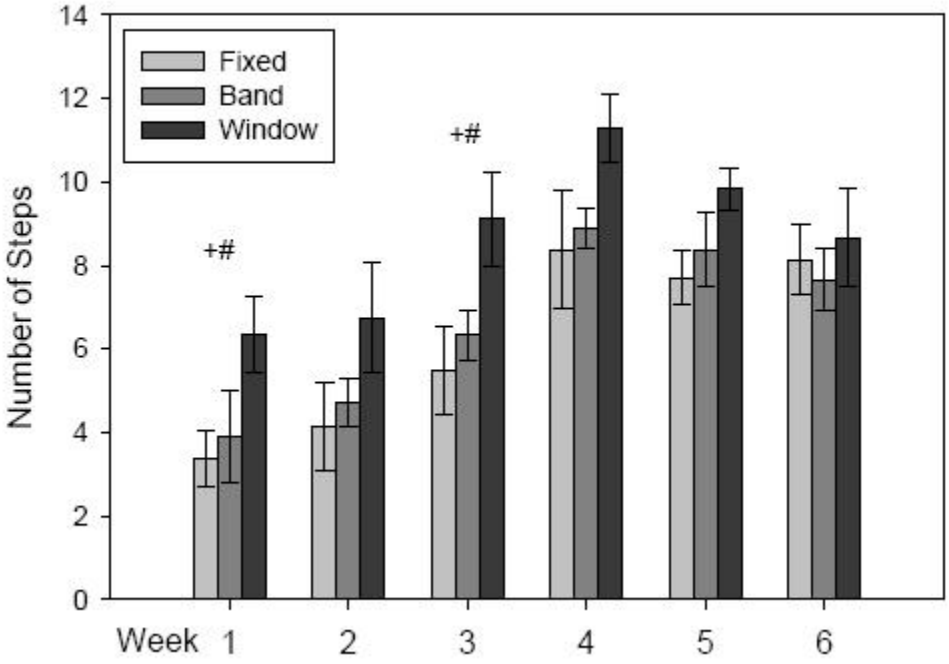

Figure 2.7: The average number of steps performed during the best 12-second interval by each of the three groups during the weekly tests. After four weeks of training, all three groups showed a significant increase in the average number of steps taken compared to week 1. On average, mice in the Window group performed better compared to the other two groups. "+" denotes significant difference between the Window and the Fixed group; "#" denotes significant difference between the Window and Band group.

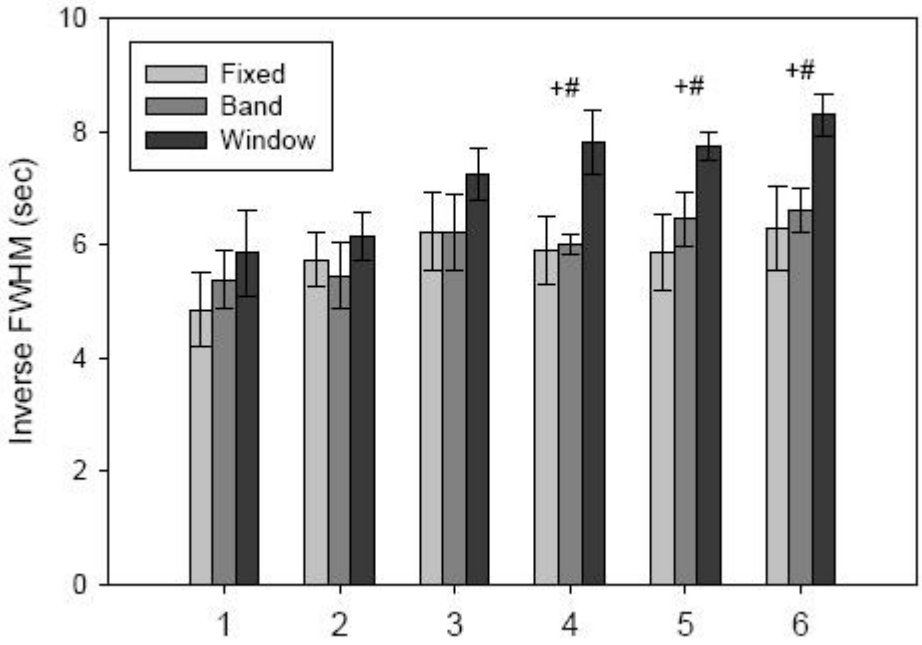

Figure 2.8: Step rhythmicity as depicted by the plot of the inverse FWHM. Although the mice trained with the Window algorithm performed more consistently than the Fixed and Band groups, the differences are not significant until the fourth week. "+" denotes significant difference between the Window and the Fixed group; "#" denotes significant difference between the Window and Band group.

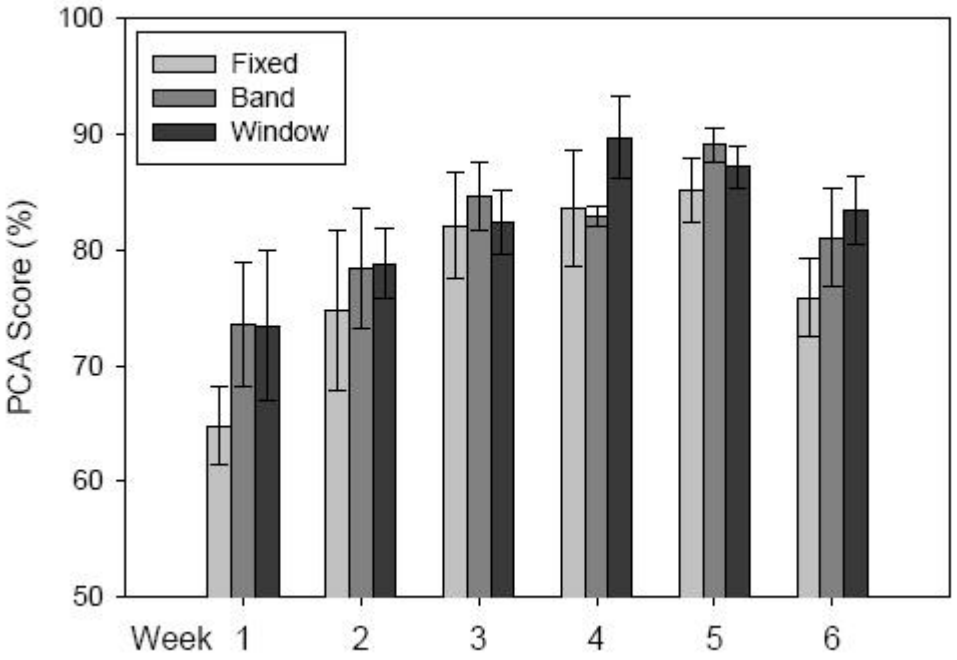

Figure 2.9: Step shape consistency as measured by PCA. There were no significant differences among the three groups at any of the weekly tests.

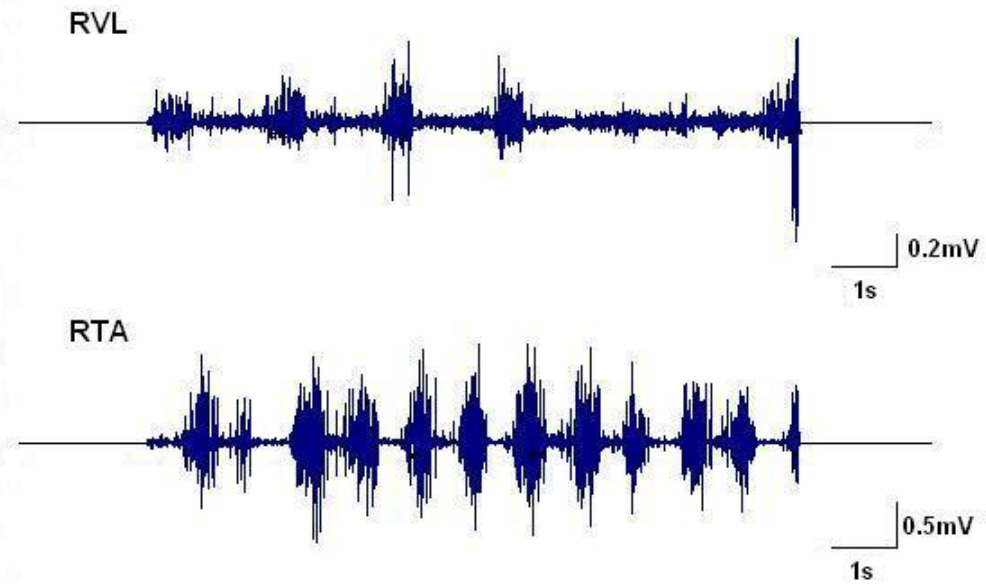

Figure 2.10: The EMG responses of fixed trajectory training. The upper plot (RVL) is from right vastus lateralis (a knee extensor) and the lower plot (RTA) is from right tibialis anterior (an ankle flexor).

EMG response to fixed trajectory training and window training was studied later in another similar experiment. Here we present the 2 best EMG responses obtained during training. In Figs. 2.10 and 2.11, the best EMG responses in fixed trajectory training and window training are shown respectively. One can easily notice the difference between the rhythmicity in the responses, especially in the vastus lateralis. In figure 2.11, the EMG of vastus lateralis (a knee extensor) is much more rhythmic than that in figure 2.10. These data suggest that the fixed trajectory actually limited the motor activity while some variability allows more natural motor output.

2.6 Discussion

The result further confirmed that combined treatment of quipazine administration and robotic training can significantly improve the stepping performance of adult spinal mice [7]. More importantly, this study showed that introducing variability into step training is more effective for locomotion recovery than fixed trajectory training. Although the fixed trajectory training has tightly controlled periodicity during train-

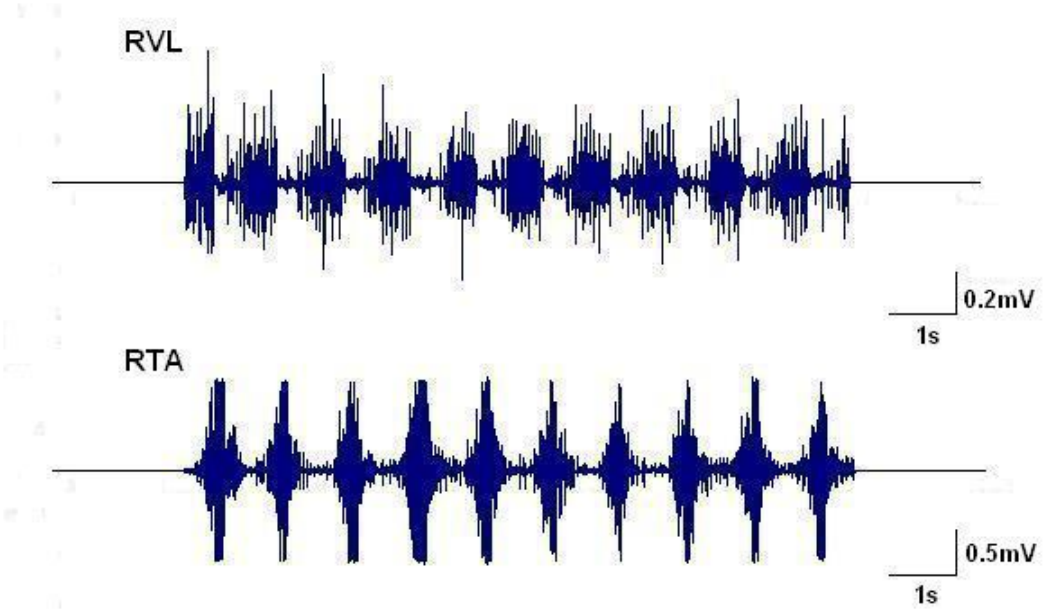

Figure 2.11: The EMG responses of window training. The upper plot (RVL) is from right vastus lateralis (a knee extensor) and the lower plot (RTA) is from right tibialis anterior (an ankle flexor).

ing, figure 2.8 shows that the fixed trained mice showed less temporal consistency than those trained with flexible paradigms, especially the window trained animals. This result, combined with the number of steps (figure 2.7), shows that the window paradigm induced better improvement in the stepping ability than the band and fixed training paradigm.

During motor learning, sensory information plays a critical role. Some specific sensory pathways are reinforced through repetitive training after SCI [22, 23]. However, variability, is a fundamental feature in the activity patterns of motor neuron circuits persists during repetitive training. Thus, a training paradigm that imposes a fixed trajectory is very unlikely to match the subsequent motor output and, therefore, the ensemble sensory information generated by the training is incongruous with the output information. This incongruity seems likely to hinder the spinal cord to learn stepping after a SCI.

On the other hand, a training paradigm that allows some variability in the output of the motor circuits could generate ensemble sensory information that is more

congruous and thus would optimize the training effect. One can imagine that there is a huge space of variability and the AAN algorithms tested in this experiment are very probably not the optimal algorithms. But one can draw a certain conclusion from this experiment that variability plays an important role in spinal cord motor learning after SCI. Further modeling and experimental work is needed to determine the optimal algorithm.

The difference between the Band and window group (Figs. 2.7 and 2.8) also showed the importance of coordination between the left and right legs in step training. The ensemble sensory information generated by a coordinated training pattern is by all means more natural than that by a noncoordinated pattern. This further confirmed the critical role of appropriate sensory information during motor learning.

One notices that the difference between shape consistency among the training groups (figure 2.9) is not as significant as the step numbers and periodicity. One hypothesis is that the PCA score may not be a good measure of the stepping quality between different training groups. Another hypothesis is that quipazine has more effect on this aspect of stepping ability than does training.

2.7 Summary

In this chapter, the effect of allowing variability in stepping kinematics while training was tested. The result shows that when variability is eliminated by the fixed training trajectory, the learning of stepping is suboptimal as compared to the condition when there is some variability during the training cycle. This implies that variability might be an important feature of the neural control by producing more appropriate ensemble sensory information for motor learning. It also gives practical direction in the further exploration of more effective robotic training device.

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Chapter 3

The Prototype Stand Platform

The work of this chapter was presented in the IEEE/RAS-EMBS Intern. Conf. Biomed. Rob. Biomechatron., 2006 as a poster, coauthored with L. Cai, J.W. Burdick and V.R. Edgerton.

3.1 Introduction

Weight bearing standing is an important function for daily life. Paralyzed people would love to restore their weight-bearing standing ability so that they could carry out some daily activities such as washing dishes in their kitchen or standing in a social situation. Also, standing appears to involve a less-complex neuromuscular model than stepping. In both senses, stand rehabilitation is an issue worthy to pursue.

Robotic devices have become more and more common in SCI rehabilitation. For example, the Lokomat is a commercially available robot for the rehabilitation of SCI and stroke patients. Robots can provide training consistency that can not be matched by therapists. And they can significantly reduce human effort during rehabilitative training. Also at the same time, robotic devices can provide precise quantification of the performance of the subjects after training. Being controlled by computer, the training program can be easily modified to suit different subjects. The training can also be adapted to the patient's progress. Thus finding an effective robotic training paradigm has attracted considerable interest of researchers [1, 2]. Bearing this in mind, we developed this robotic training system for standing rehabilitation in adult

spinal mice and rats. It could serve as a prototype for future human applications.

3.2 The Stand Platform

3.2.1 Design Motivation

As mentioned in Chapter 1, stand rehabilitation is potentially of great practical significance. Stand rehabilitative training has been carried out on spinal cats [3] and rats [4]. Cutaneous inputs from the hind paws have been shown to be important in the locomotion control in spinal and intact cats [5, 6]. In stand training, cutaneous inputs were also used as the stimuli to trigger the standing behavior [3].

Due to the small body size of mice (body length of 2 inches), it is difficult to find a commercially available yet versatile enough device to train and test the spinal mice. Thus we built a 6-DOF parallel manipulator with a plate end effector (figure 3.7), which theoretically is able to move along any trajectory within its work space (for our device, its workspace is approximately a cube with 2 in sides). It was shown in our study that the mechanical device works well for mice and rats. With the hind paws of the animal on the moving plate and the forepaws supported by a fixed plate, we can stimulate the mice with different kinds of mechanical stimuli, such as vertical up and down, side-to-side translation, and tilt. so as to provide the spinal cord with load and stretch stimuli during standing.

After the complete spinal cord transection, a mouse cannot support its body weight at all. Body weight support is therefore needed to assist the animal to initiate a standing posture. How much weight the subject can bear by itself is an obvious measure of the training effect. With the performance of the subject changing, we wish to adjust the level of weight support adaptively and be able to measure the amount of weight support. With this in mind, our system can actively adjust the amount of weight support under computer control. With the feedback of the animal performance, an adaptive weight support schedule can also be applied during the training process.

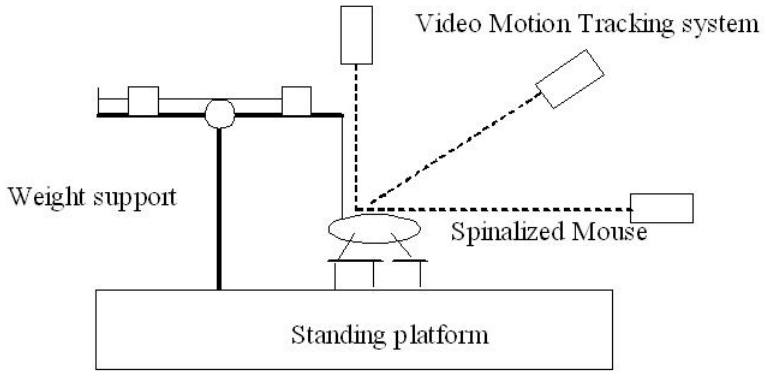

Figure 3.1: The schematic of the robotic stand training system.

The response of the animal, including the joint angles, EMG signals of major muscle groups, and ground reaction force, can be recorded by a video tracking system, EMG electrodes, and load cells that measure plate reaction forces.

In the following section, details of the system design are given.

3.2.2 System Overview

figure 3.1 shows the schematic of our system. The whole system is composed of a stand platform, an active weight support system and a 3-camera motion tracking system. In figure 3.2, a mouse is sitting in the system with weight support attached to its sacrum. During training, the hind feet of the mouse are put on the moving plate of the platform and the fore feet are on a fixed support. In our preliminary experiments, two suture loops are implanted under the sacrum connection tissue of the mice. Weight support is provided by hooking the mice to the weight support system through the implanted suture loops. (A wisely designed harness could be more convenient than implanting suture loops in each mouse.) Then different stimuli can be given to the hind limbs when the moving plate moves along a prescribed trajectory. The active support system provides a certain level of weight support during the training. The motion tracking system can monitor the movement of a marker attached to the sacrum of the mouse.

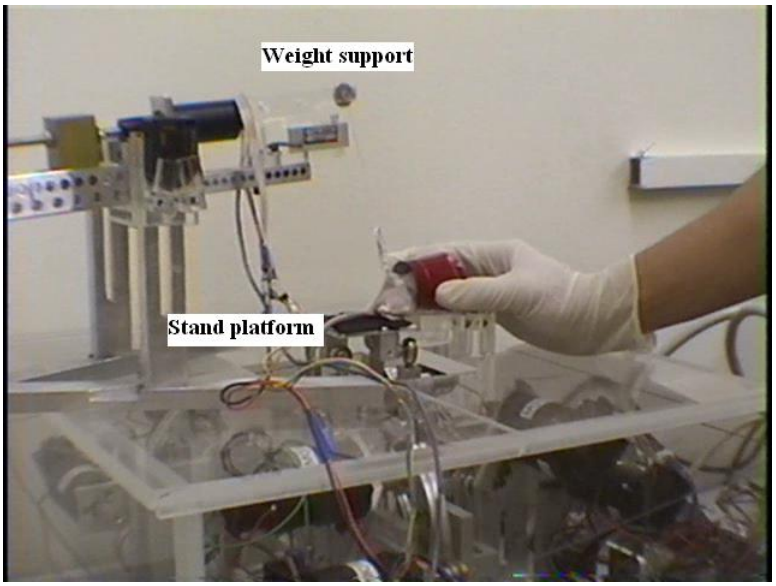

Figure 3.2: The robotic training system with a mouse. The motion tracking system is not displayed in this photo.

3.2.3 Stand Platform

A six degree-of-freedom (DOF) platform is built as the main training device. The platform is a parallel manipulator driven by four robotic linkages. The kinematic configuration is shown in figure 3.3. The platform is modeled after the "NINJA" parallel manipulator in [7] because it has a compact configuration and potentially high moving speed and acceleration.

In the mechanism, each linkage has two active revolute joints and one passive revolute joint which is perpendicular to the two active ones. Parallelogram linkages are used here for the arrangement of the motors, as can be seen in the linkage model in figure 3.4. The platform is actually a redundant system since 3 of the four linkages are enough for generating the 6-DOF motion of the plate. But for the sake of mechanical balancing and the simplicity of trajectory planning, four identical linkages arranged symmetrically are used to drive the platform (figure 3.3). A plate is attached via spherical hinges onto the top of the four linkages.

To get a high performance on velocity and acceleration, the friction resistance between the mechanical parts should be minimized. For this purpose, 8 DC Motors (3863H024C DC motors combined with HEDL5540 optical encoders, MicroMo Elec-

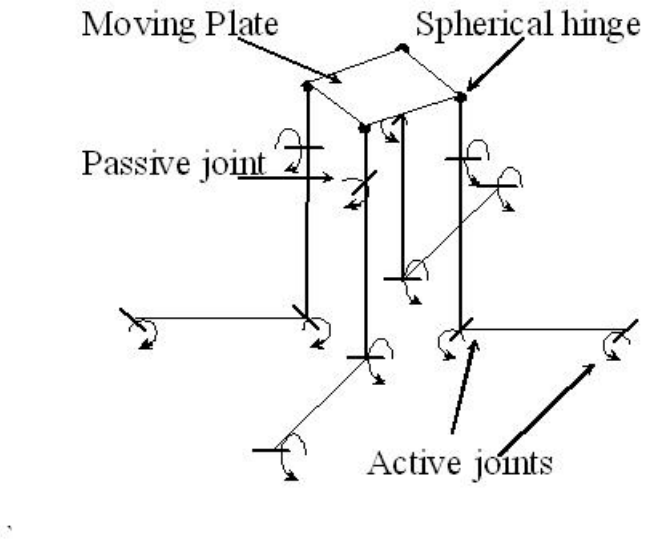

Figure 3.3: Schematic configuration of the 6-DOF platform.

tronics, Inc.) drive the linkages through cable-driven speed reducers (figure 3.5). The cables activate through tension so that there is almost zero friction as oppose to normal dog-tooth gears, and they are also maintenance free, i.e., free of lubrication. The motors are controlled by an 8-axis PCI Galil Motion Control board (Galil 1800, Galil Motion Control, Rocklin, CA). The joint trajectories are precalculated by solving the inverse kinematics along desired training trajectories.

The platform has 6 DOFs to enable arbitrary training trajectories. This allows us to be able to try different stimuli and test the training effect with different types of disturbances.

3.2.4 Weight Support Systems

The weight support system (figure 3.8) has a balance weight whose position can be adjusted through a screw drive, which is controlled by a DC motor. A load cell (model GS0-50 from Transducer Techniques with a measurement capacity of 50 grams) is used to monitor the tension in the cable which the nmouse hangs from. An optical encoder is mounted on the pivot axis of the top lever to measure the tilt

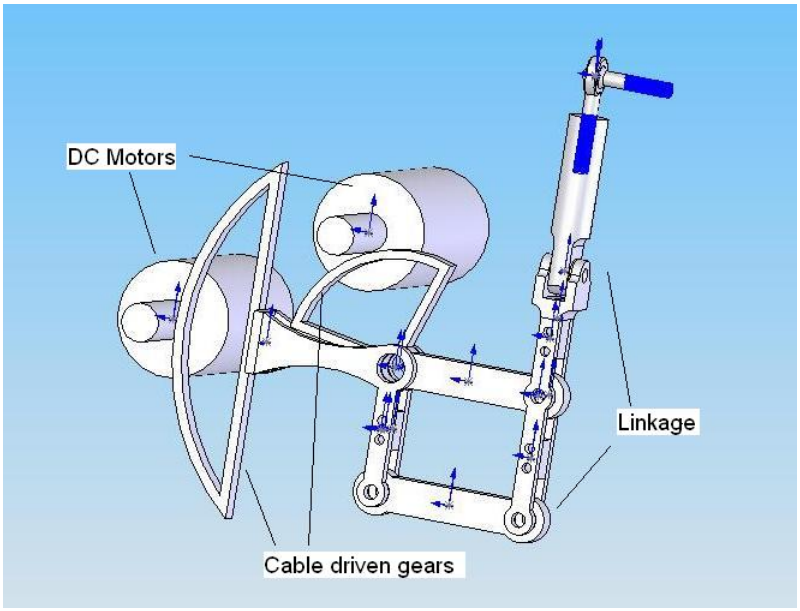

Figure 3.4: The SolidWorks model of a driving Linkage with two DC motors.

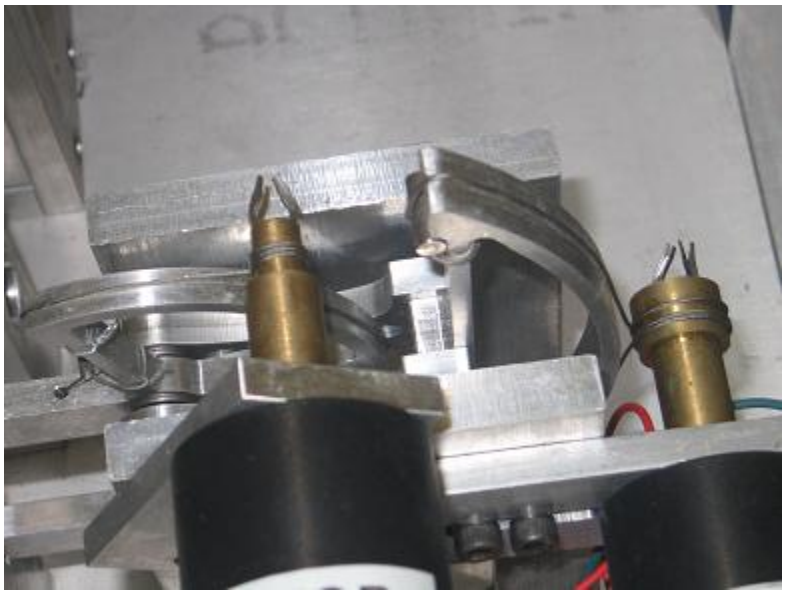

Figure 3.5: A snapshot of the cable-driven gears.

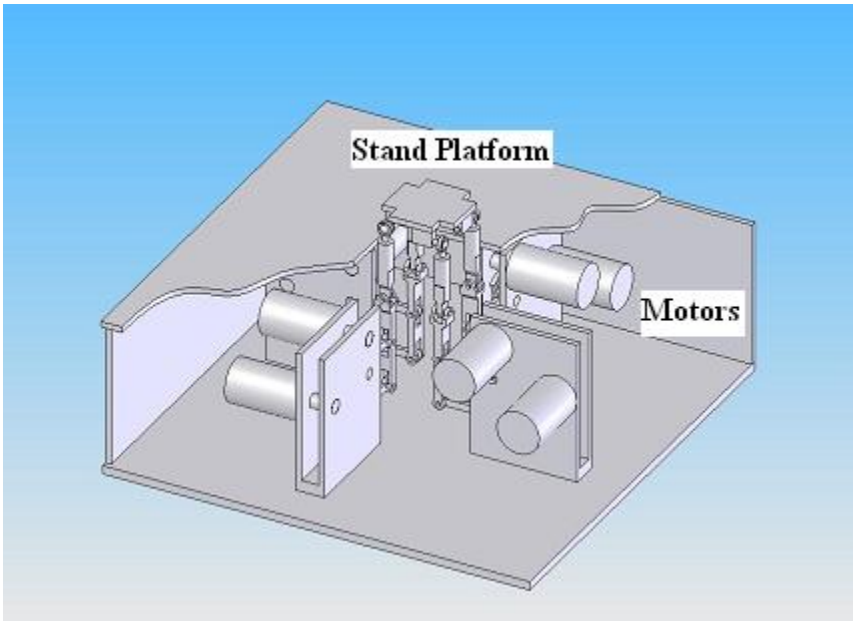

Figure 3.6: The SolidWorks model of platform.

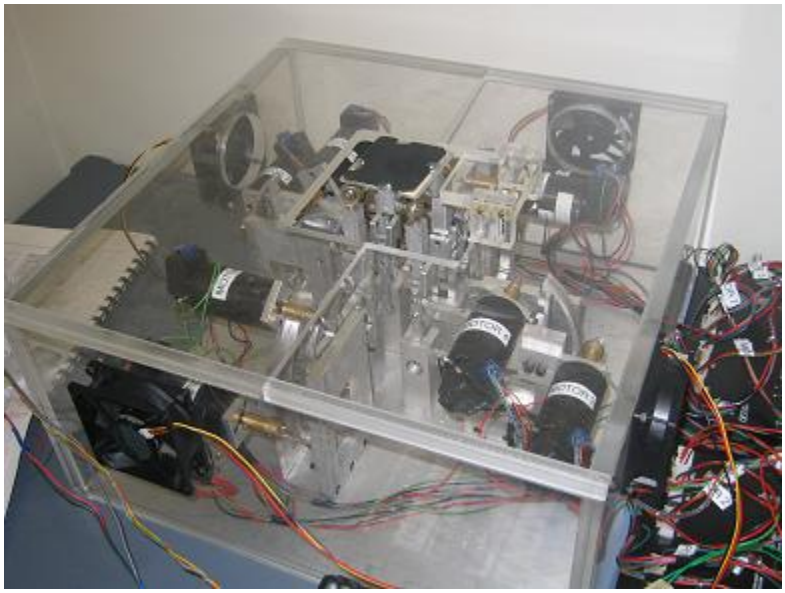

Figure 3.7: The photograph of the stand platform.

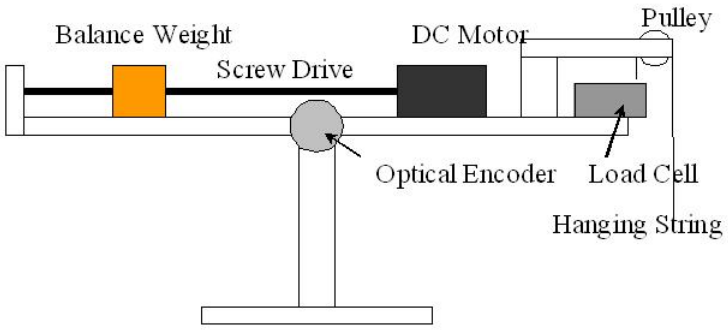

Figure 3.8: The schematic diagram of the active weight support system.

angle, from which we can infer the mouse posture. With the load cell readout as the feedback information, the motor is able to adjust the position of the balance weight and thus provide active body weight support to the mouse. The load cell can also provide additional information to quantify the weigh support ability of the mouse. The weight support system is controlled by a 4-axis PCI Galil Motion Control Board (Galil 1840, Galil Motion Control, Rocklin, CA).

To work with rats, another weight support system was developed and used in the final study. The schematic diagram is shown in figure 3.10

During training, the rat is put into the weight support harness and maintains a posture of quadrupedal stance. A passive spring element is connected to the hind hanging point of harness through cable to provide the main unloading force to the hind body. The amount of body weight support can be measured by the force sensor. The front hanging point connected to the horizontal beam directly. Potentially, if the other end of the spring is connected to a DC motor, then an active closed-loop can be formed so that the weight support level can be adjusted by moving the end position of the spring.

3.2.5 Performance Measurement

To monitor the response of the mouse during training (mainly the position of the sacrum point), a 3D motion tracking system is built to track the motion of a marker

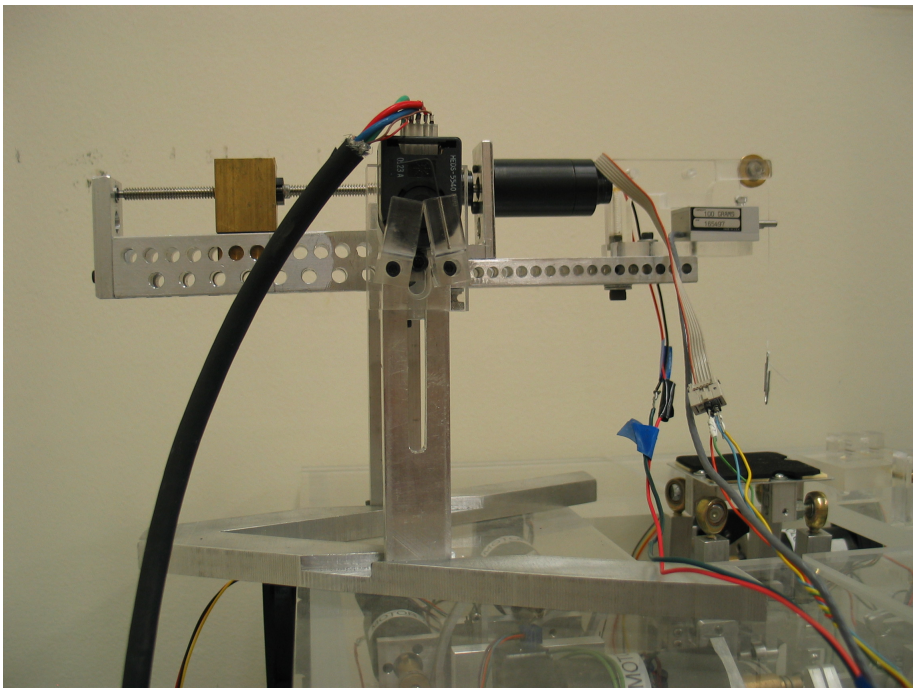

Figure 3.9: The active weight support system.

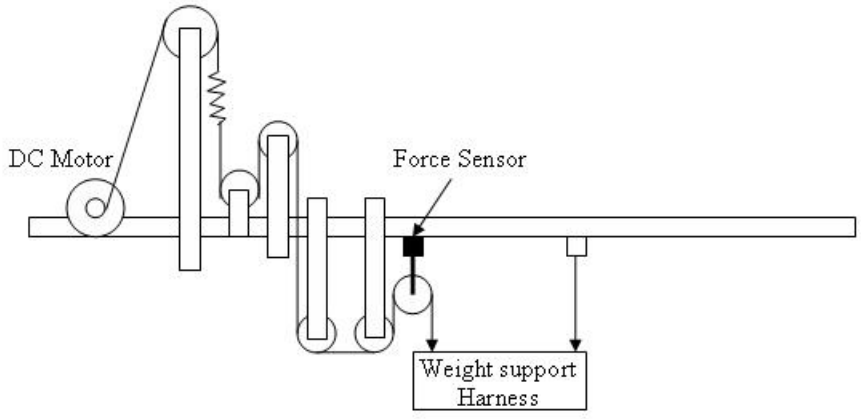

Figure 3.10: The schematic diagram of the rat weight support system.

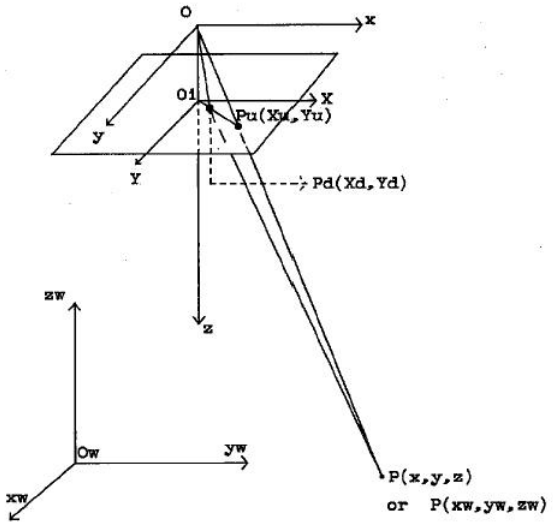

Figure 3.11: Camera geometry with perspective projection and radial lens distortion (modified from [8]).

attached to the hip point. Three noninterlaced CCD cameras (MC-CC-P60 60 fps progressive scan "Cube Cam," resolution 659 × 497 pixels) and an multichannel frame grabber (PC2Vision from Dalsa Coreco) are used to record the motion of the marker point at a frequency of 30 Hz. Progressive scan can eliminate the motion blur. A real-time image processing program can then provide the 3D coordinates of the marker point, which give us the base point of the hindlimbs. Tsai's method [8] is employed for the camera calibration and motion tracking.

The basic geometry of the camera model is shown in figure 3.11. The variables (x_w, y_w, z_w) denote the 3D coordinate of the object point P in the 3D world coordinate system. The variables (x, y, z) denote the 3D coordinate of the object point P in the 3D camera coordinate system, which is centered at point 0, the optical center, with the z-axis the same as the optical axis. The variables (X_u, Y_u) denote the image coordinate system centered at O_i (intersection of the optical axis z and the front image plane) and parallel to x- and y-axis. f is the distance between front image plane and the optical center. (X_u, Y_u) is the image coordinate of (x, y, z) if a perfect pinhole camera model is used. (X_d, Y_d) is the actual image coordinate which differs from (X_d, Y_d,) due to lens distortion.

The transformation from the 3D world coordinate to the camera coordinate is

defined as follows:

$$\begin{bmatrix} x \\ y \\ z \end{bmatrix} = R \begin{bmatrix} x_w \\ y_w \\ z_w \end{bmatrix} + T, \tag{3.1}$$

where R is the 3×3 rotation matrix

$$R = \begin{bmatrix} r_1 & r_2 & r_3 \\ r_4 & r_5 & r_6 \\ r_7 & r_8 & r_9 \end{bmatrix}, \tag{3.2}$$

and T is the translation vector

$$T = \begin{bmatrix} T_1 \\ T_2 \\ T_3 \end{bmatrix}. \tag{3.3}$$

The parameters to be calibrated are R and T.

Then the 3D camera coordinate (x, y, z) is transformed to the ideal (undistorted) image coordinate (X_u, Y_u) using the pinhole camera geometry

$$X_u = f\frac{x}{z}, \tag{3.4a}$$

$$Y_u = f\frac{y}{z}. \tag{3.4b}$$

The parameters to be calibrated here is the focal length f.

The radial lens distortion is modeled as

$$X_d + D_x = X_u, \tag{3.5a}$$

$$Y_d + D_y = Y_u, \tag{3.5b}$$

where (X_d, Y_d) is the distorted coordinate on the image plane and

$$D_x = X_d(k_1 r^2 + k_2 r^4 + \cdots), \tag{3.6a}$$

$$D_y = Y_d(k_1 r^2 + k_2 r^4 + \cdots), \tag{3.6b}$$

$$r = \sqrt{X_d^2 + Y_d^2}. \tag{3.6c}$$

The parameters to be calibrated are the distortion coefficients k_i.

Real image coordinate (X_d, Y_d) is transformed to computer image coordinate (X_f, Y_f) by the following

$$X_f = s_x d_x^{'-1} X_d + C_x, \tag{3.7a}$$

$$Y_f = d_y^{-1} Y_d + C_y, \tag{3.7b}$$

where (X_f, Y_f) are the row and column numbers of the image pixel in computer frame memory; (C_x, C_y) are the row and column numbers of the center of computer frame memory; d_x is the center to center distance between adjacent sensor elements in X direction;

d_y is the center to center distance between adjacent sensor elements in Y direction;

$$d_x' = \frac{N_{cx}}{N_{fx}}, \tag{3.8}$$

where N_{cx} is the number of sensor elements in X direction and N_{fx} is the number of pixels in a line as sampled by the computer. The parameter to be calibrated is the uncertain image scale factor s_x.

By combining the above equations and keeping only the first-order radial distortion, the computer coordinate (X_f, Y_f) is related to the world coordinate of the object

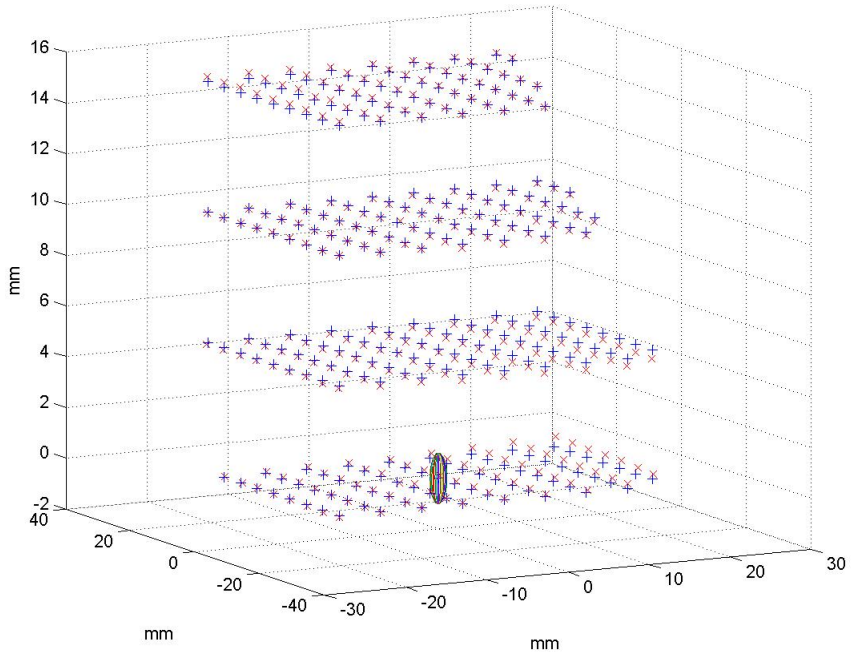

Figure 3.12: Calibration of the 3-camera motion tracking system. In this figure, the points represented by the "+" signs are the calibration points with known space positions. Those "x" points are reconstructed points after the cameras are calibrated. A unit sphere is plotted at the origin to serve as a scale.

point by the following equation:

$$s_x^{-1} d'_x (1 + k_1^2)(X_f - C_x) = f \frac{r_1 x_w + r_2 y_w + r_3 z_w + T_x}{r_7 x_w + r_8 y_w + r_9 z_w + T_z}, \tag{3.9a}$$

$$d_y (1 + k_1^2)(Y_f - C_y) = f \frac{r_4 x_w + r_5 y_w + r_6 z_w + T_y}{r_7 x_w + r_8 y_w + r_9 z_w + T_z}, \tag{3.9b}$$

where the r_is are elements of the rotation matrix in (3.2). The parameters to be calibrated are R, T, f, k_1, s_x, (C_x, C_y).

Using an array of points with known positions in the space, the unknown parameters can be calibrated. After the parameters are calibrated, the world coordinate of a point can be reconstructed by solving equations (3.9) gotten from independent cameras. figure 3.12 shows the reconstructed position of an array of spatial points.

The 3-camera motion tracking was developed originally to use with the mouse weight support system. However, the rat weight support takes more space and blocks

the view fields of the cameras.

Ground reaction force sensors can be mounted on the moving plate of the platform. So the force applied to the hind paws during the experiment can be recorded.

EMG electrodes can be implanted in the major extensor and flexor muscle groups during experiments. By comparing the EMG behavior, hindlimb kinematics, and ground reaction force generated by different stimuli, some relations between stimulation and standing rehabilitation can be determined, which in turn may further reveal the mechanism underlying the full-weight-bearing stand.

3.3 Preliminary Experiments in Spinal Mice

Preliminary experiments were conducted to test the capacity of the stand platform. More detailed experiments which test the hypothesis about stand rehabilitation are presented in the next chapter.

The animal protocol has been presented in the previous chapter. Briefly, Adult female Swiss-Webster mice (body weight around 25 g) obtained from Charles River Laboratories (Wilmington, MA) were used. A complete mid-thoracic spinal cord transection was performed at around 60 days of age.

Simple pilot studies were carried out to test the system's capacity. Sinusoidal and trapezoidal (duty cycle of 30%) vertical perturbations (figure 3.13) with magnitude of 2 mm and frequencies of 1, 2 and 3 Hz were given to the mice with or without quipazine (a 5HT agonist which shown to be able to enhance the training effect in stepping [9]). Results showed that among the stimuli we tried, the 2 Hz trapezoid perturbation triggered the most response of the mice, which, in terms of the joint angle, is an increased angle between the paw and the support surface. figure 3.14 shows a comparison before and after training. Note the difference between the angle of the ankle. When quipazine was administrated, the mice had a stronger response to the stimuli.

The preliminary study confirmed the capacity of the platform. The animals could be properly positioned in the device and the weight support system worked well

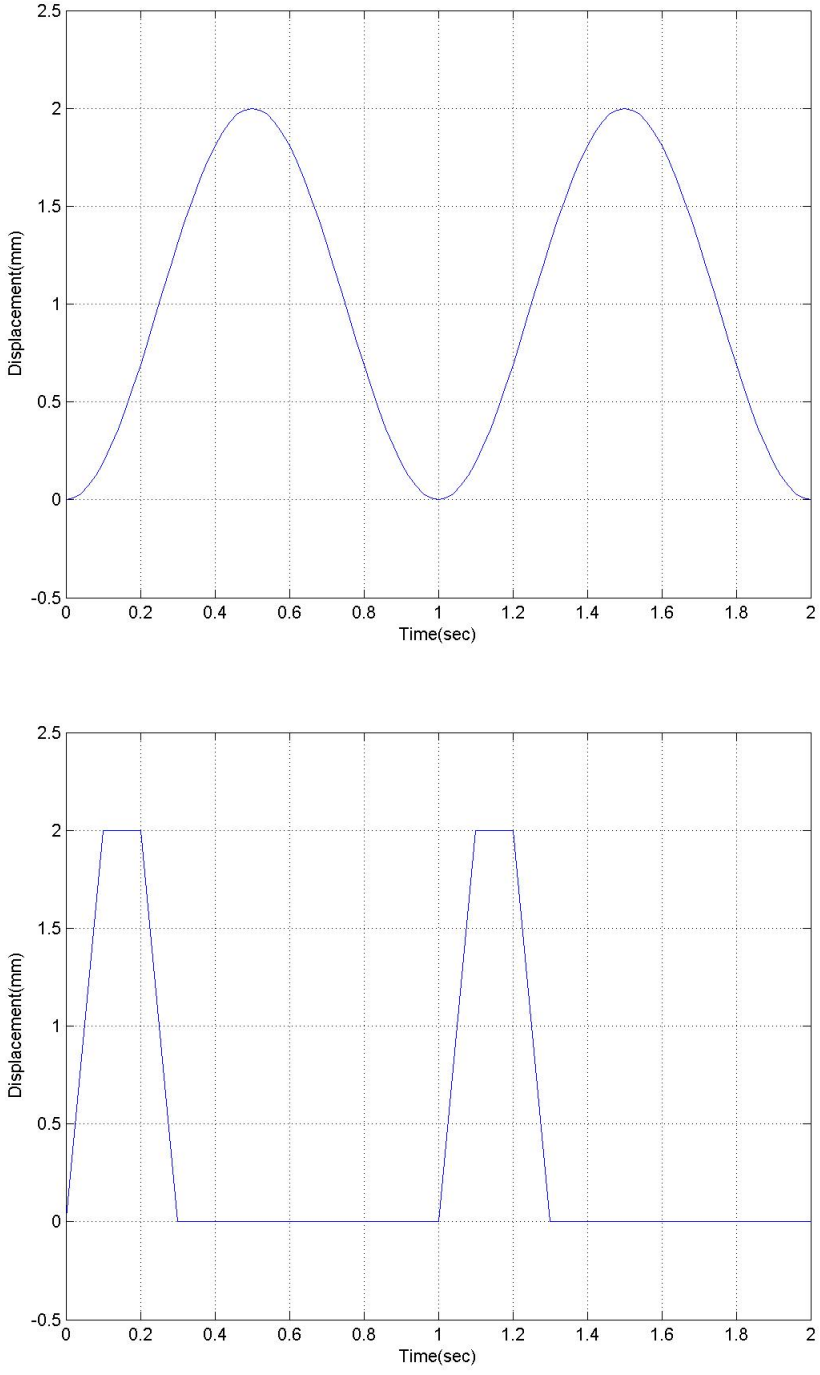

Figure 3.13: Examples of the perturbation signals.

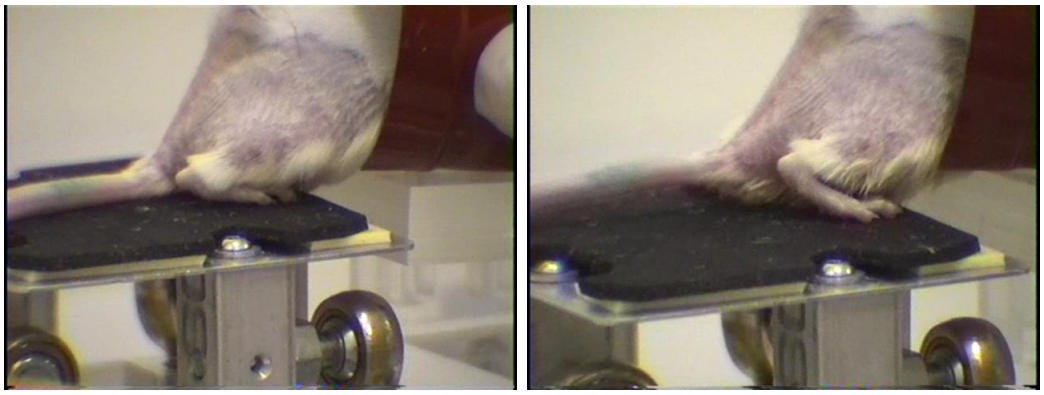

Figure 3.14: The snapshots of the hindlimb before and after training. Note the difference between the angles of the ankle joint.

enough for the training.

3.4 Summary

In this chapter, a prototype platform for standing rehabilitation study was developed and preliminary experiments were conducted in mice. The experiments showed that the platform worked well and was able to generate different stimulation to induce responses in mice. In the following chapter, more detailed study in adult spinal rats carried out with the platform will be presented.

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Chapter 4

Standing Rehabilitation

4.1 Introduction

As mentioned in previous chapters, step training has received extensive attention and has been conducted in many animal models and human subjects. After intensive training, animals with complete spinal cord injury can recover some stepping ability [1, 2]. These results suggest that the spinal cord circuitry has a great degree of automaticity and plasticity after an injury. The spinal cord is able to perform a motor task, e.g., stepping and scratching, without supraspinal input. And some of the potential of the spinal cord is referred to as "learning" specific motor tasks that are practiced.

The task dependency of locomotion learning in spinal cord implies that the plasticity of the spinal cord is sensory modulated [1, 3]. For example, during step training, the sensory information of stepping is generated by moving the limbs along a stepping trajectory and delivered to the spinal cord circuitry. With this afferent input, the spinal cord undergoes a series of changes so that after a period of time. Specific sensory pathways are reinforced and the spinal cord can generate stepping output when provided with appropriate sensory input triggered by the treadmill moving backward.

Standing is another important motor function besides stepping in everyday life. Prior research has shown the feasibility and efficacy of step training. While in the mean time, relatively less attention was paid to standing rehabilitation after spinal cord injury despite its clinical and scientific significance. Clinically, if a paraplegic

patient can stand, there will be a great improvement in his or her everyday life. Everyday tasks that require a standing posture, such as washing dishes in the kitchen, standing and talking in a bar, can be performed. Also there may be great health benefits such as improved blood pressure maintenance, reduced incidence of skin lesions, and reduced loss of bone density in the legs. All these have the potential to change the psychological outlook and well-being of the individual.

From the scientific point of view, study of standing rehabilitation after SCI can provide more insights into the mechanism of motor control. It has been suggested that the postural control and locomotor control systems might share common neuronal structures at the subcortical level (brainstem-spinal cord system) from studies in decerebrated cats [4]. Experiments in cats showed that those cats trained to step could not stand well, while those trained to stand had poor stepping performance [3]. However, it is not clear whether these two tasks are controlled by two separate circuits or not. Further study in standing and stepping rehabilitation is needed.

Postural control is a complex process and involves different parts of the central nervous system [5, 6, 7]. Postural adjustment was studied in intact animals [8, 9, 10]. And the roles of pyramidal tract and reticulospinal system in postural corrections were also studied in cats [11, 12].

Postural muscle tone plays an important role in the fulfilment of posture and locomotion movements, such as standing up and stepping. Stimulation of certain parts of the brainstem can modulate the muscle tone and thus change the posture and locomotion status. For example, electrical stimulation on the MLR (Mesencephalic Locomotor Region) evokes walking; electrical stimulation on the DTF (Dorsal part of the Tegmental Field) changed the posture from standing to sitting and sitting to lying while electrical stimulation on the VTF (Ventral part of the Tegmental Field) caused a cat to stand up [4, 13]. A critical level of muscle tone is necessary for locomotion, since walking is suppressed when tone becomes too low or too high [4].

Spinal cord injured animals seem to have primitive balance control that is due to muscle and ligament stiffness or perhaps local segmental reflexes of the spinal cord [14, 15, 16, 17]. Stand training was conducted in spinal cats [1, 3, 18]. After a period

of training, the cats could stand for a much longer time than those cats that received no training [3], and the inhibitory capacity (GAD_{67} levels) in the spinal cord was different between trained and non-trained animals [18]. Stand training has also been performed on human SCI individuals too, and it was shown that the training induced functional changes in the spinal cord [1, 19]). Subjects are able to bear more limb load after stand training, producing a higher EMG output. Also the trained limb produced higher EMG during stepping though it did not result in overall improved locomotion pattern as step training [19].

During standing in normal animals, the postural muscle tone is maintained at a certain level to counteract the gravity force. And the Ia primary afferents were tonically active [20, 21]. After an SCI, the supraspinal tonic input is no longer delivered to the spinal cord. And the muscle tone is usually not sufficient to support the body weight.

It has been shown that Ia afferents input could cause sustained increase in excitability of α-motoneurons and it was due to plateau depolarization of the motoneurons (MNs) [22]. Plateau potentials are maintained by persistent inward current (PIC) [23]. These plateau potentials have an important functional role in the motoneurons [23, 24]. With the plateau potential, the MNs can maintain sustained activity with little or no tonic synaptic input [25]. Repeated stretch or contraction of muscles can also induce Ia afferents input and thus lower the threshold of plateau potential and increase the MN excitability [26, 27, 28, 29, 30]. It was observed that after a short period of repetitive stimulation, the EMG lasted longer with each stimulation and sustained firing occurred in some motor units [26, 27]. This suggested a short-term plasticity in the motoneuron properties, i.e., the repeated synaptic input has a residual effect in some membrane properties so that PIC could occur at lower thresholds [28, 30].

The success of step training implies that repetitively performing a task can cause long-term plastic change in the spinal cord. In step training, afferent information associated with stepping is delivered to the spinal cord repetitively and as discussed before, the spinal cord neuronal circuits undergoes some long term plastic change.

Analogously, we propose to examine the effects of repetitive training on the recruitment of the extensor motoneurons after an SCI.

To study the standing rehabilitation after SCI, we developed the standing platform which was described in detail in the previous chapter. This 6-DOF parallel platform is able to move along a precalculated trajectory under the control of computer. With this device, we can generate different kinds of mechanical stimuli to the animal. For example, loading and unloading to the hind limbs can be generated when the platform moves up and down vertically when the animal is put on top of the platform.

4.2 Experimental Study

4.2.1 Animal Protocol

In the experiment, adult female Sprague-Dawley rats from Charles-River Laboratories (Wilmington, MA) were used. The rats were housed in spacious cages individually, with access to food and water ad libitum, and were kept on a 12-hour light/dark cycle for the duration of each day. All animal procedures were conducted in accordance with the Animal Care Guidelines of the American Physiology Society and were reviewed and approved by the Animal Research Committee at the University of California at Los Angeles.

4.2.2 Surgical Procedures

Surgeries were performed at approximately postnatal day 60. The rats were anesthetized with Ketamine 23 mg plus Xylazine 1.0 mg combo. Supplements of 30% of the initial dosage were given every 1.5 hours through out the surgical time. During surgery, a water circulating heating pad was used to maintain body temperature.

Spinal transection and EMG electrodes implantation were performed under aseptic conditions. A skin incision was made along the sagittal suture of the skull. The scalp musculature and underlying connective tissue was reflected laterally and the exposed skull was dried thoroughly. Three screws were anchored firmly to the skull

and one Omnetic 12-pin connector (head plug) was cemented (using dental cement) to the skull and screws. Eight Teflon-insulated stainless steel multistrand wires (Cooner AS 631-2) were led subcutaneously from the connector to the right and left leg. Skin incisions were made in the hindlimb to expose the soleus (Sol) and tibialis anterior (TA) muscles. A pair of wires was inserted to each muscle region (∼2-3 mm apart) by passing them individually through a 25-gauge hypodermic needle. Recording electrodes were made by removing ∼0.5 mm of the insulation from each wire. Following back-stimulation of the muscle through the head plug to ensure the proper placement of the electrodes, each lead was secured with a suture at its entry and exit from the muscle. This procedure effectively secured the electrodes in the muscle belly. The bared tips of the wires were covered by gently pulling the Teflon coating over the tips to avoid recording extraneous potentials. The fascia surrounding the muscles were closed using 4.0 Dexon suture and skin incisions were closed using 4.0 Ethilon suture. An additional wire was embedded in the middle back region and serves as a common ground. For the spinal cord transection, a skin incision was made along the dorsal midline from approximately from T7 to T9. A laminectomy was performed and a complete spinal transection was made as spinal level T8. The completeness of the transection was verified by two surgeons by gently lifting the proximal ends of the spinal cord. The transected area was cleaned and gel foam was inserted between the proximal stumps of the spinal cord to prevent regrowth. The connective around the opened area was sutured and then the skin incision was closed.

Right after surgery, the rats were placed in an incubator maintained at ∼29 ± 1 °C and observed until they fully recovered from anesthesia.

4.2.3 Post Surgical Care

Baytril (40 mg/kg body weight), a broad spectrum antibiotic, was added to the drinking water for 14 days after surgery. During the first 3 days after surgery, Buprenex (pain killer), 0.05 mg/kg, was given to the rats through subcutaneous injection twice each day. The bladders were expressed manually 3 times each day during the first 3 days after surgery and twice daily thereafter to minimize the risk

of bladder infection and related complications. Food rewards were given to stimulate positive interaction between the animals and handler.

4.2.4 Euthanization

At the conclusion of each study, the rats were anesthetized deeply with sodium pentobarbital (50 mg/kg) and transcardially perfused with 1 mL/g body weight of 0.1 M phosphate buffer, pH=7.4, followed by 2 mL/g body weight of 4% paraformaldehyde for 12 min. The spinal cords then were removed, postfixed in 4% paraformaldehyde for 5 hours, and cryoprotected by incubating in a 30% sucrose solution in 1× phosphate buffered solution overnight to ensure adequate absorption. The cords were then frozen on dry ice, and stored at −80 °C.

4.2.5 Preliminary Study in Rats

To test the work capacity and identify an appropriate training paradigm, a pilot study on spinal rats was conducted. The purpose of training was to trigger as much motoneuron (MN) activity as possible. The activity level is reflected by the EMG response in the hindlimb muscles. Adult female Sprague-Dawley rats (body weight around 200 g) obtained from Charles River Laboratories (Wilmington, MA) were used. Following the procedures outlined in sections 5.2.2 and 5.2.3, the spinal cord was completely transected at the midthoracic (between T7 and T9) level and EMG electrodes were implanted in soleus (an ankle extensor) and TA (tibialis anterior, an ankle flexor) muscles bilaterally.

For each experiment the rat was placed in a weight support harness (See figure 4.1). Weight support was provided so that the rat could maintain a standing posture on top of the platform. The hind feet were in contact with the platform and the fore feet were in air so that the fore limbs did not bear any weight.

Muscle stretch can activate the Ia afferents. Two kinds of stimuli were tried during the pilot study. The first one is fast vertical vibration of the platform. figure 4.2 and figure 4.3 show typical EMG responses in the right (RSol) and left soleus (LSol) to

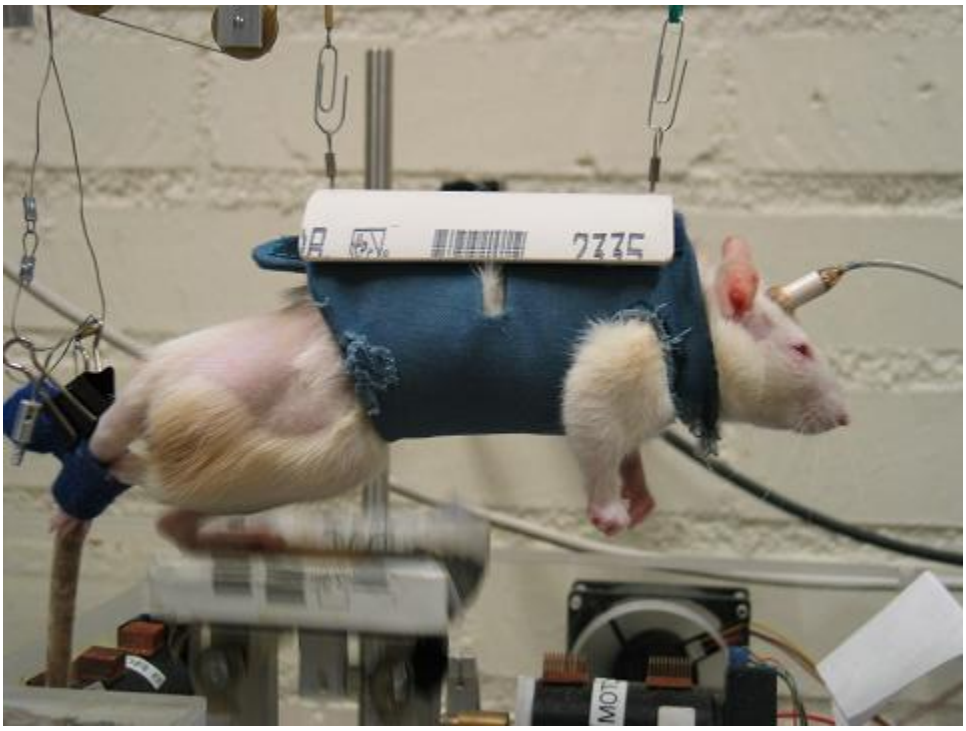

Figure 4.1: A rat being trained on the platform. One hindlimb is tied up so that the training is focused on one side. This was to eliminate the load uncertainty between the two sides.

the platform vibration. The bottom plot in the figures depict the movement of the platform. At the beginning of each platform moving cycle, a spike is generated to synchronize the EMG record with the platform movement. The vibration used here is a 10 Hz sinusoidal vibration with amplitude of 3mm.

In general, tonic EMG response could be triggered by the vibration of the platform and sometimes could remain after the vibration stops, as shown in figure 4.2. But it is not consistent with the platform movement. As seen from figure 4.3, the EMG activity in the left soleus starts and stops as the vibration goes on. Also according to observation, the rats had less EMG response after three weeks of training. The possible reason could be adaptivity to the training paradigm. Thus another paradigm was tried.

The basic idea of the second training paradigm is to repetitively load and unload the hind limbs through the vertical up and down movement of the platform. figure 4.4 shows the scheme of the periodic movement of the platform. As the platform moves

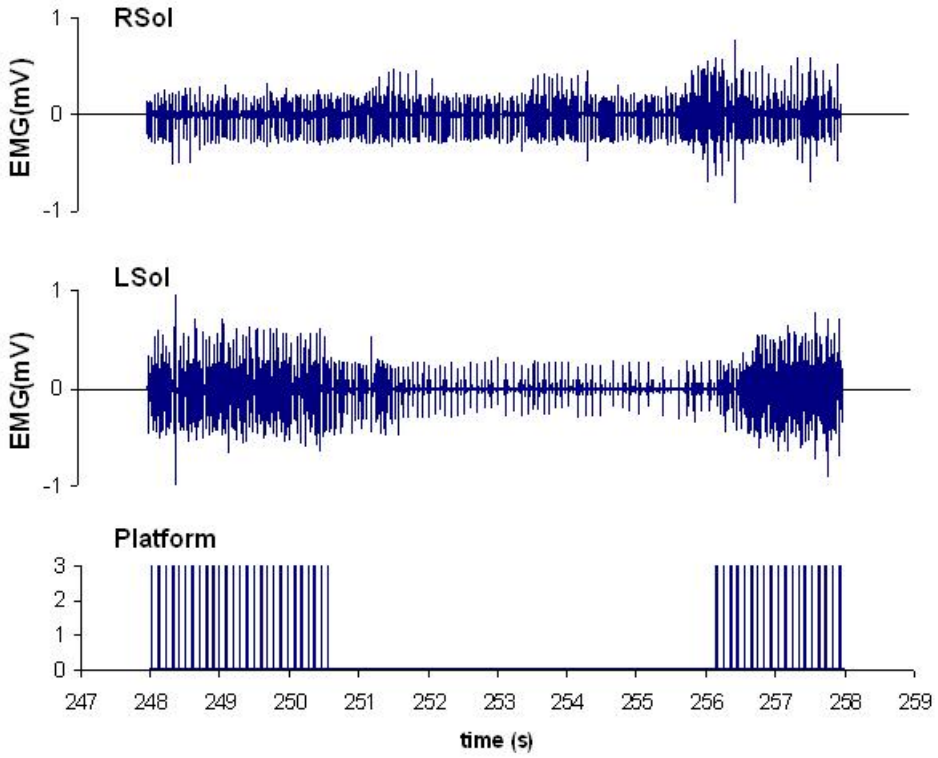

Figure 4.2: EMG responses to vertical platform vibration ($3mm$ amplitude) in right soleus (RSol) and left soleus (LSol). The spike train in the bottom plot indicates the on/off of the platform vibration. Note that the EMG remains or decays after the platform stops.

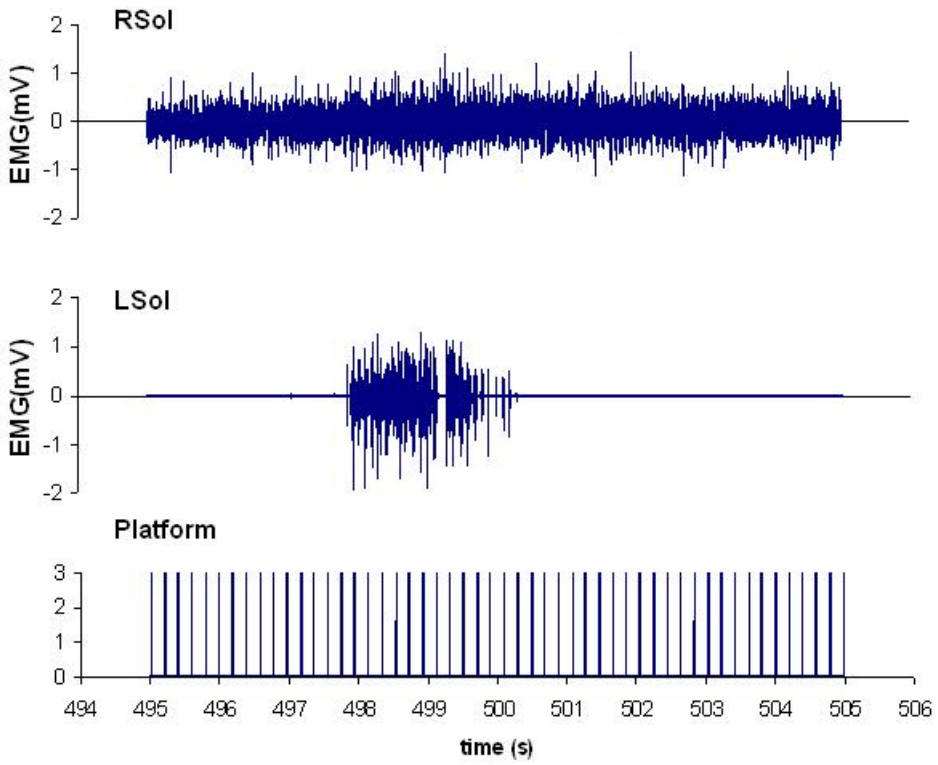

Figure 4.3: EMG responses to vertical platform vibration in right soleus (RSol) and left soleus (LSol). The spike train in the bottom plot indicates the on/off of the platform vibration. Note the asymmetry in EMG with the platform movement.

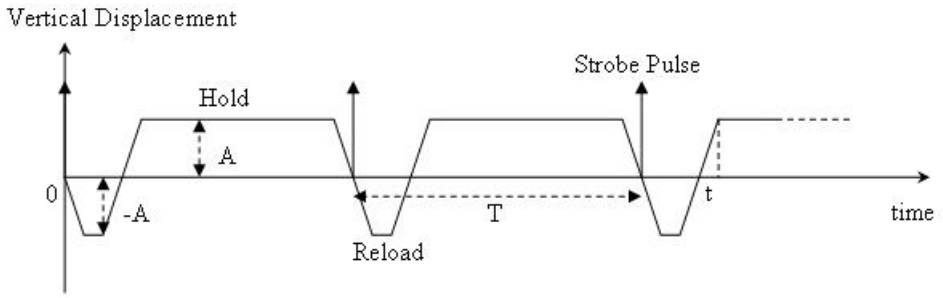

Figure 4.4: Schematic plot of the vertical movement of the platform. The computer generates a strobe spike (the vertical arrows) at the beginning of each period as a signal for synchronization between the EMG record and the platform movement.

up and down, the hind limbs are intermittently loaded and unloaded. After holding for a certain period, the platform lowers briefly and then moves up again to generate another loading to the hind limbs. The major parameters of the trapezoidal wave are the amplitude A, the period T and the time t taken for the platform to move from neutral position to the up or lower limit positions.

figure 4.5 shows a typical response at the beginning of training. During each load and hold cycle, the EMG response has dynamic (when the platform moves up) and static components (during the holding phase). It can be observed that after some loops of loading and unloading, the static response lasts longer and longer, i.e., there is a short-term windup effect [26, 27].

As the training continued, more static EMG responses were triggered. figure 4.6 shows a typical response when the training had lasted for a period of time. Note the well-sustained EMG activity following each platform perturbation.

After the platform stopped, the EMG could remain for different periods of time (figure 4.7 and figure 4.8).

To determine the appropriate values of A, T and t, a series of tests were conducted in 4 rats. First, the amplitude A was varied. Based on the range of motion of the hind limbs, a value of $A = 15mm$ (denoted by A_m below) which was below the full range of motion was decided, with which the hind limbs can be suppressed to a certain

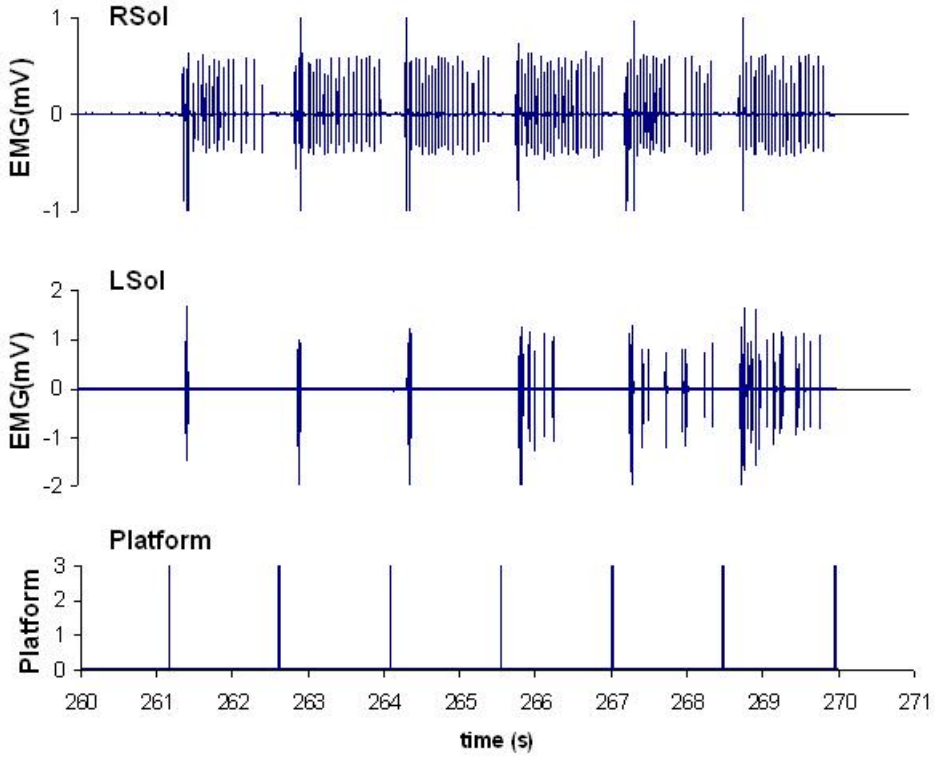

Figure 4.5: EMG responses in right soleus (RSol) and left soleus (LSol) to repetitive loading/unloading recorded from the beginning of a training session. Note the windup of the EMG activity.

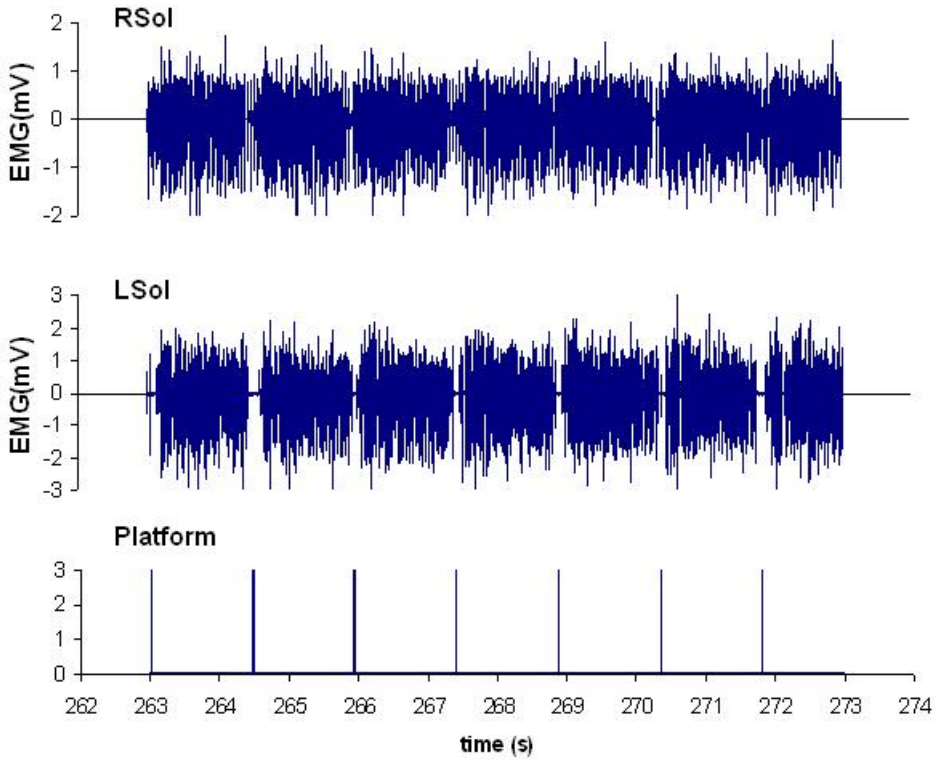

Figure 4.6: Sustained EMG responses in right soleus (RSol) and left soleus (LSol) to repetitive loading/unloading occurred in the middle of a training session.

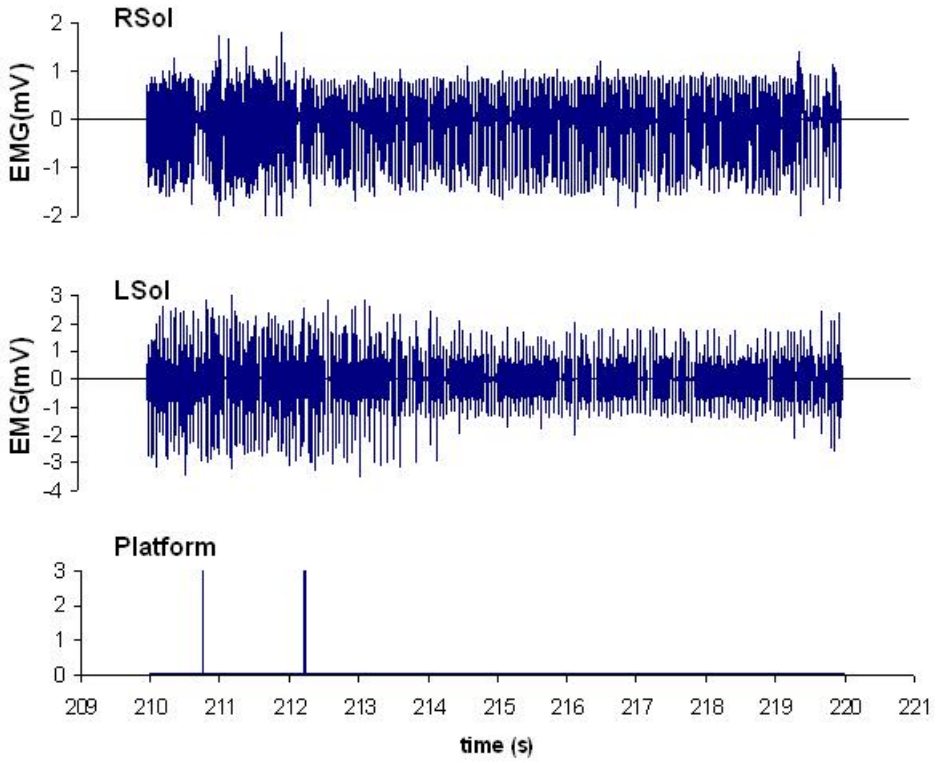

Figure 4.7: EMG responses in right soleus (RSol) and left soleus (LSol) well-sustained after training in some rats.

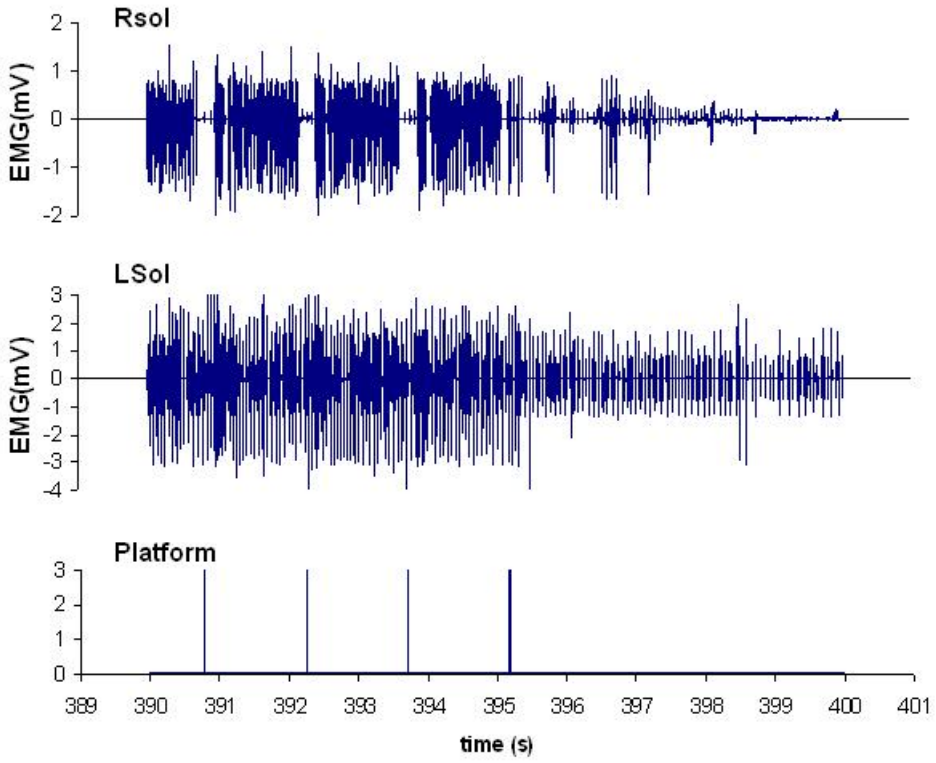

Figure 4.8: Another example of sustained EMG responses in right soleus (RSol) and left soleus (LSol) after training.

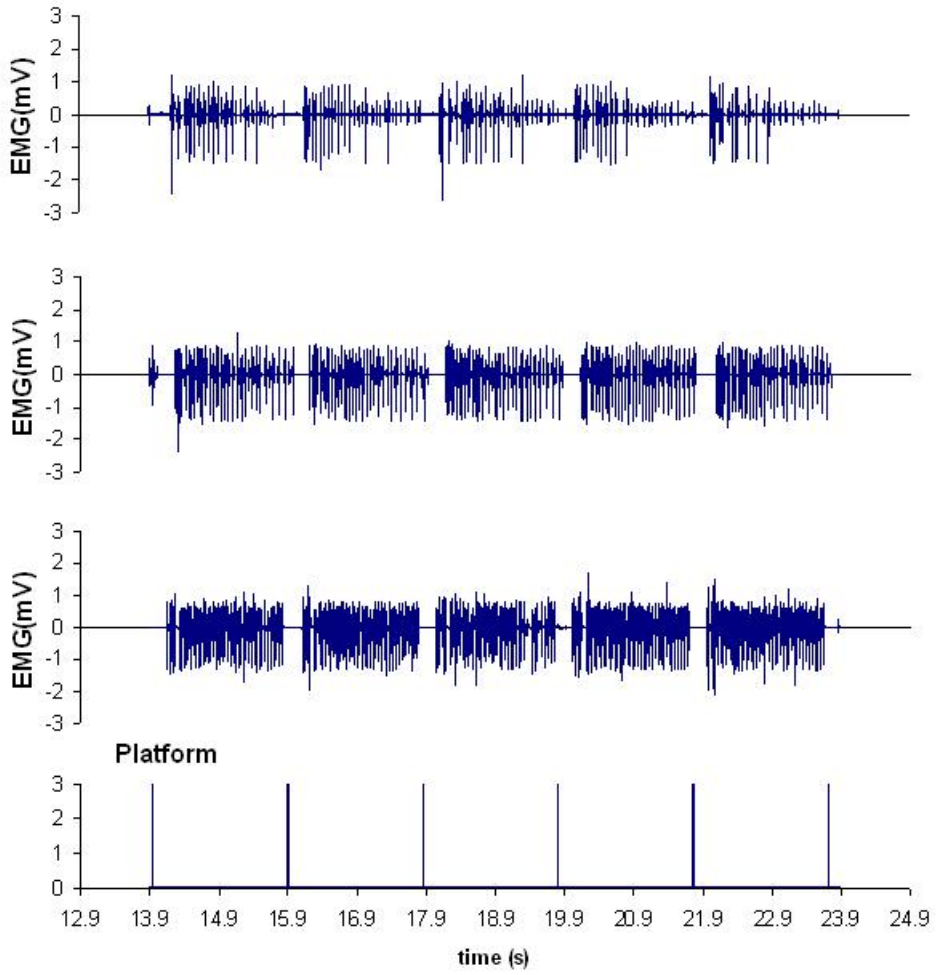

Figure 4.9: EMG responses in soleus to stimulations of different amplitudes of $0.75mm$, $11.25mm$ and $15mm$ from the top down. The bottom plot indicates the movement of the platform. Note that EMG response increases with the amplitude.

degree but not totally collapsed. And another two values of $0.75A_m$ and $0.5A_m$ were chosen to test the effect of loading amplitude. Typical EMG responses at different amplitudes are shown in figure 4.9. The EMG responses increase with the amplitude of displacement (figure 4.10). In the final training, a value of $15mm$ of platform vertical displacement was selected.

EMG responses were also studied with different time intervals (T took $1s$, $2s$, $4s$). Typical responses are shown in figure 4.11. The T effect was not as significant as the amplitude (A) (figure 4.12). So in the final training, $2s$ intervals were selected although $1.5s$, $3s$, and $4s$ intervals also resulted in a sound EMG response. In general,

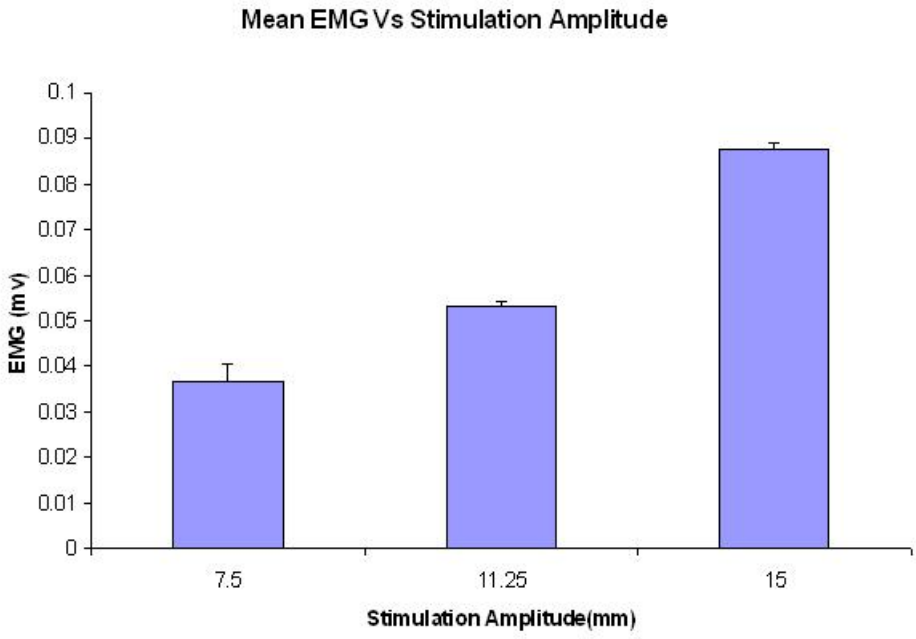

Figure 4.10: Mean EMG amplitudes (mean ± SE) increase with stimulation amplitudes. The average was based on 10 consecutive stimulation periods.

a $2s$ interval resulted in the most robust and continuous response.

The effect of loading time t ($0.04s$, $0.08s$ and $0.16s$) was also tested and the results are shown in figures 4.13 and 4.14. A faster loading tends to trigger better EMG response.

Based on the above tests, in the final training, the following parameters were selected: $A = 15mm$, $s = 0.04s$ and $T = 1.5s$, $2s$ or $3s$ adjusted to achieve the most robust EMG response.

To test the ability of postural adjustment of the spinal cord, the response to lateral tilting of the platform (figure 4.15) was tested. Two typical records are shown in figure 4.16 (α follows a sinusoidal wave pattern, maximum angle 20 degrees, right side moves up first in each cycle) and figure 4.17 (α follows a trapezoidal wave pattern, maximum angle 20 degrees, right side moves up first in each cycle). A general conclusion that can be made is that the EMG activity is well correlated to the stretch loading of the muscle, which is the same as observed in [17]. However, there was little evidence of active lateral posture adjustment in the lower spinal cord.

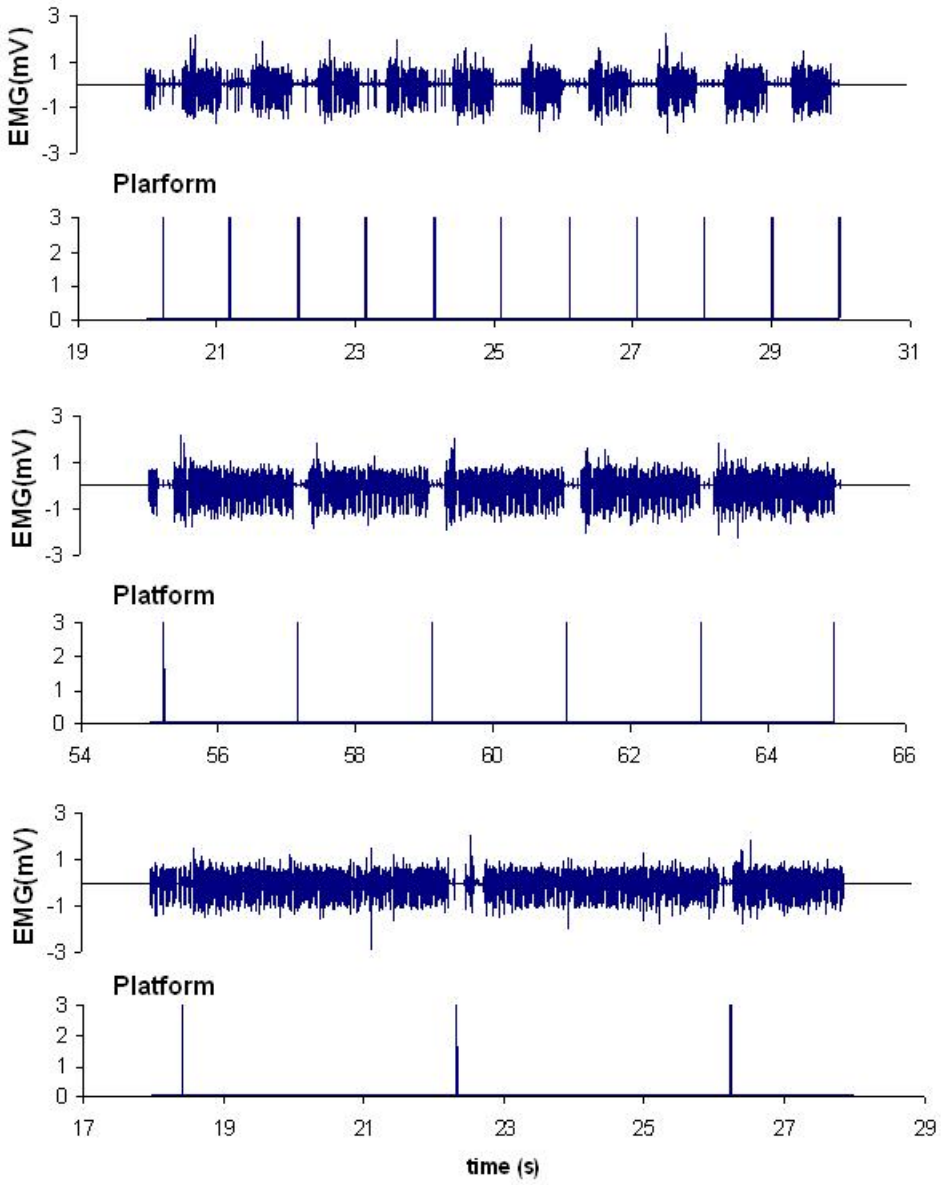

Figure 4.11: EMG responses to stimulations of $T = 1s$, $T = 2s$ and) $T = 4s$ from top down.

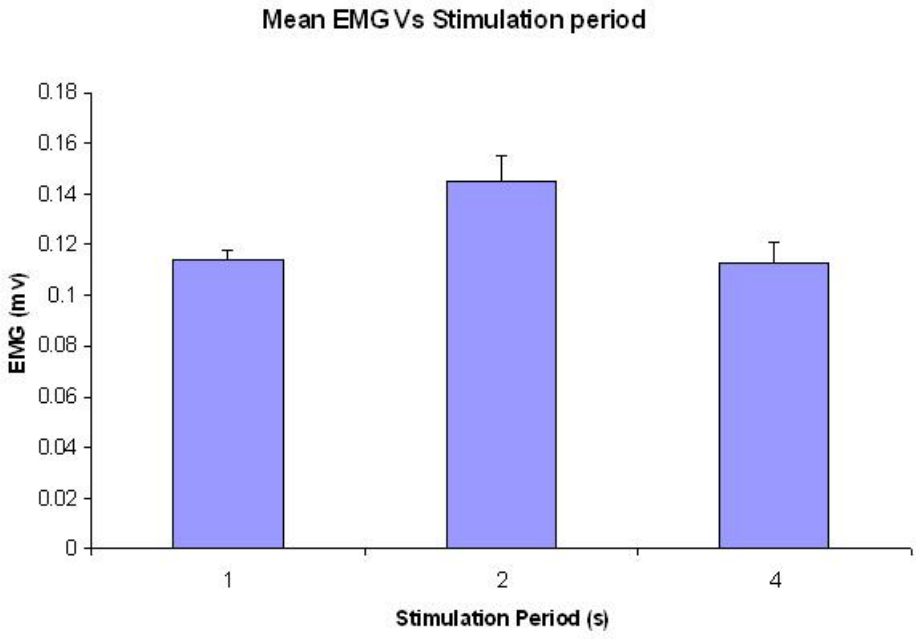

Figure 4.12: Mean EMG amplitudes (mean ± SE) with stimulations of different periods. The average was based on 10 consecutive stimulation periods.

EMG responses to platform perturbation were recorded during testing with and without bicuculline (a GABA antagonist which reduces the inhibitory level of GABA in the spinal cord). The percentage of mean EMG amplitude change in the soleus in the presence or absence of bicuculline is plotted in Figures 4.18 and 4.19.

In most cases, the EMG response in the soleus was improved with bicuculline if the untreated response was small. So during training, bicuculline was administrated to all the animals.

4.2.6 Stand Training

The aim of this study was to characterize the chronic effect of daily robotic training on the neuromuscular plasticity of the extensor response after spinal cord transection. The rats were randomly divided into two groups, one control group and one trained group. A pilot study showed that bicuculline, a GABA antagonist, could increase the EMG response induced by load perturbation in a given test session. So in the final study, bicuculline was administrated (IP, intraperitoneal injection) to both groups of

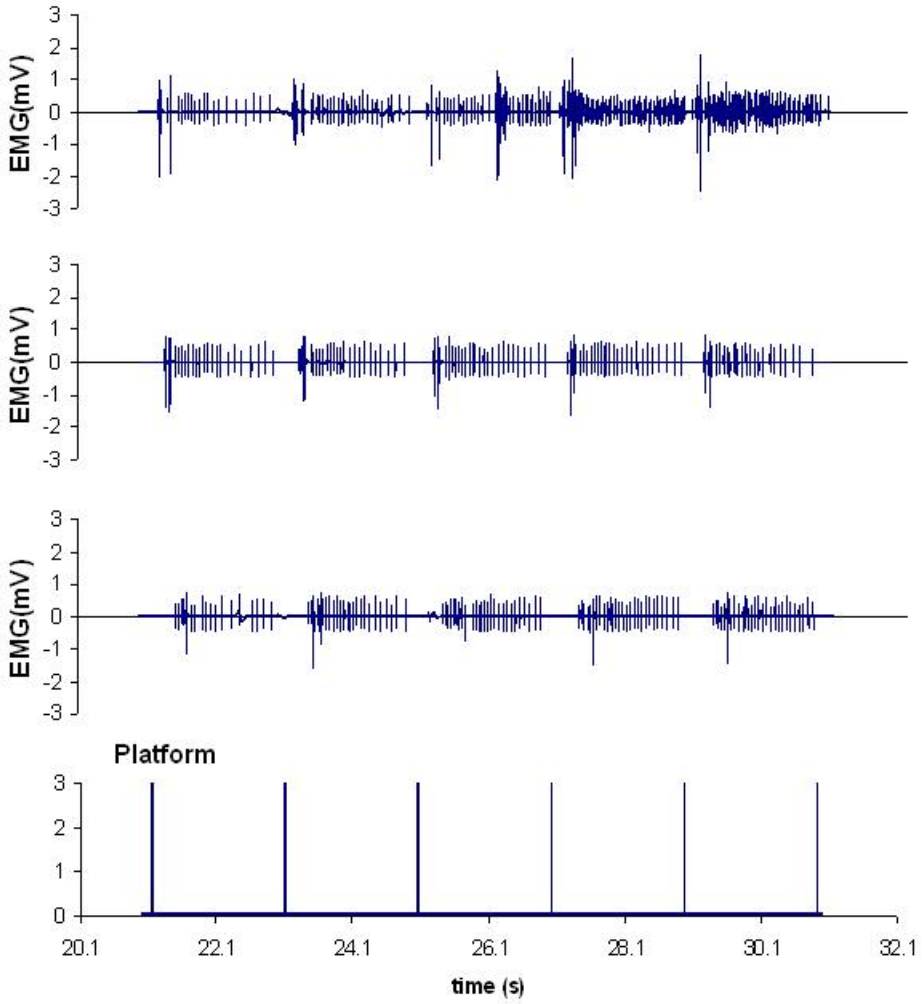

Figure 4.13: EMG responses to stimulations of different loading time: $t = 0.04s$, $t = 0.08s$, and $t = 0.16s$ from top down. Generally the shorter loading time for a given amplitude of displacement, the greater the EMG response.

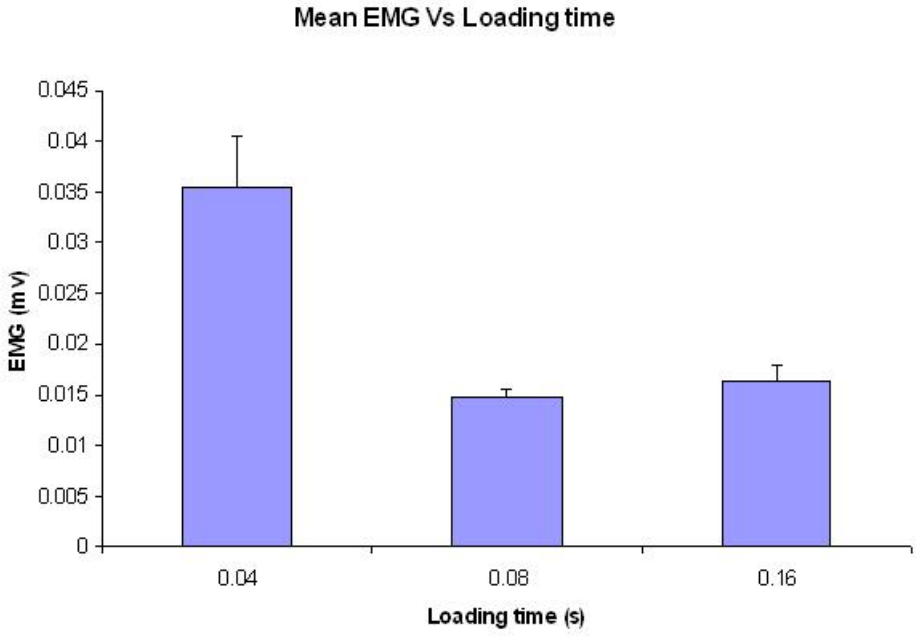

Figure 4.14: EMG amplitudes (mean ± SE) with stimulations of different loading time. The average was based on 10 consecutive stimulation periods.

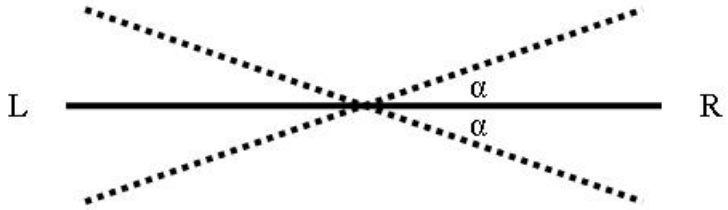

Figure 4.15: Scheme of the lateral tilting movement of the platform

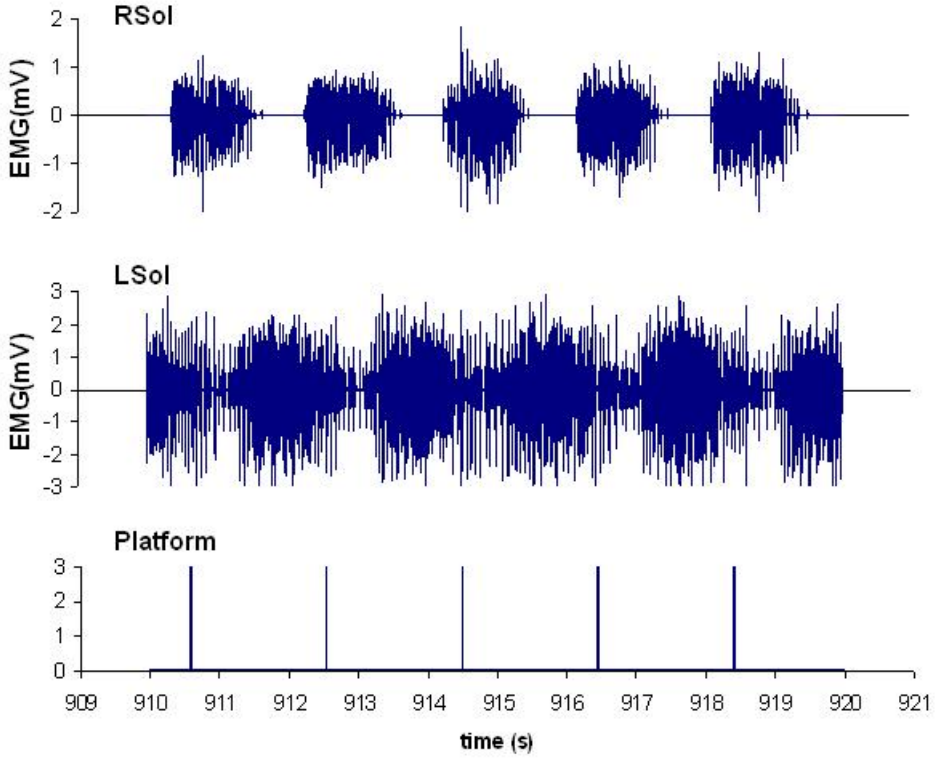

Figure 4.16: EMG responses to sinusoidal tilting of the platform in right soleus (RSol) and left soleus (LSol). The spikes in the bottom plot indicate the starting point of the sinusoidal tilt.

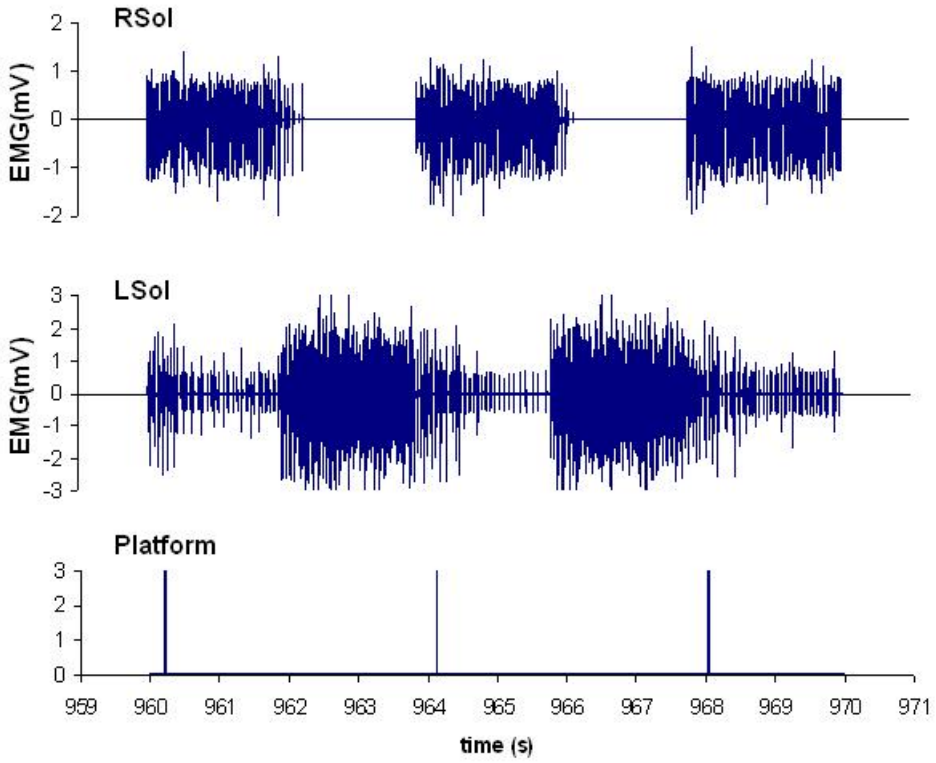

Figure 4.17: EMG response to trapezoidal tilting of the platform in right soleus (RSol) and left soleus (LSol). The spikes in the bottom plot indicate the initiation of the trapezoidal tilt.

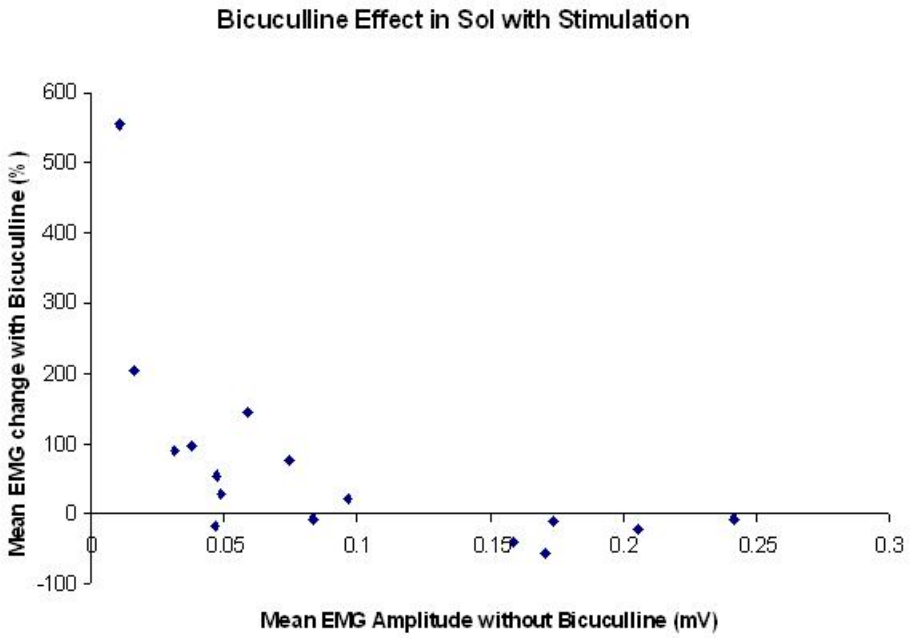

Figure 4.18: Effect of bicuculline during stimulation. The y-axis is the percentage change of EMG activity in soleus in presence of bicuculline relative to that in absence of bicuculline (x-axis). Each data point depicts the response from one rat (16 in total).

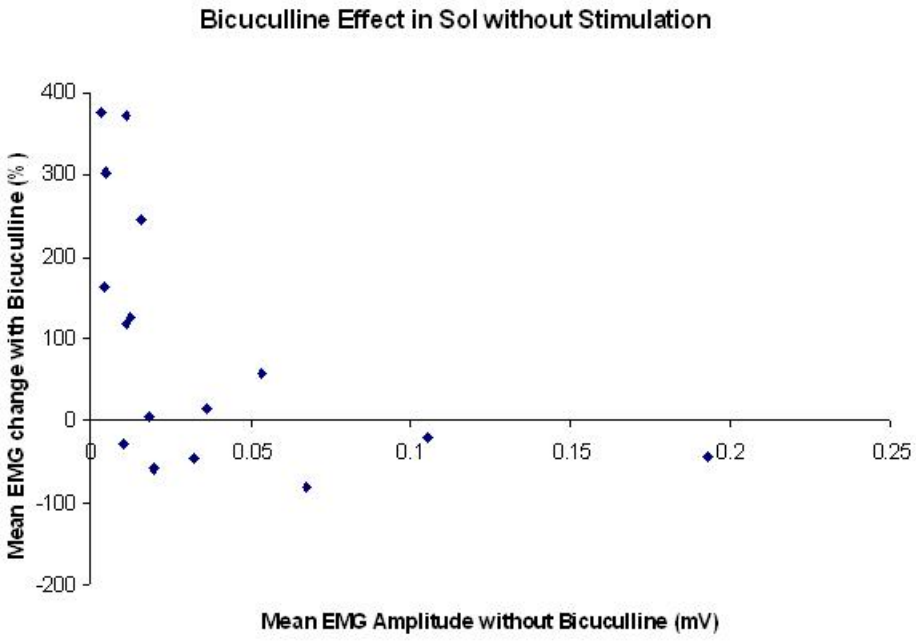

Figure 4.19: Effect of bicuculline at rest. The y-axis is the percentage change of EMG activity in soleus in presence of bicuculline relative to that in absence of bicuculline (x-axis). Each data point depicts the response from one rat (16 in total).

trained and non-trained rats. The hindlimb of the rats in the train group was placed on the platform, beginning 10 min after of injection to receive training. The training lasted for 10 min. The non-trained rats in the control group were treated the same as those in the train group except that the platform remained unperturbed.

During training, the rats were put into a weight-support harness and oriented in the posture of quadrupedal standing (figure 4.1). The hind feet were in contact with the platform and the fore feet were not allowed to bear weight. Then the platform moved up and down vertically. The Y trajectory is the trapezoidal wave illustrated in the prior section (figure 4.4).

The training was performed everyday Monday through Thursday. On Fridays, the animals were tested. The test took the scheme shown in figure 4.20. The rat was first stimulated by the platform for 2 minutes (referred to as stimulation period) and then the platform stopped and kept still for 1 minute (referred to as rest period). In the stimulation period, the platform move in the trapezoidal fashion shown in figure 4.4.

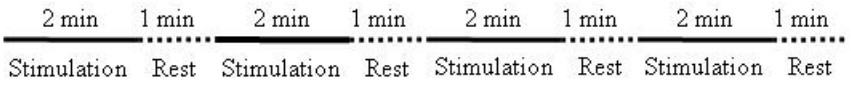

Figure 4.20: Standing test scheme.

Table 4.1: The p-values of the ANOVA test of normalized simulation EMG in soleus between trained and control groups

Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
0.0634	0.3954	0.3905	0.8444	0.6032	0.6454

with $T = 2s$, $A = 15mm$ and $s = 0.04s$. The stimulation/rest cycle was repeated 4 times in each test session. The rectified EMG response was integrated for each stimulation and rest periods. Then the overall average EMG for the stimulation and rest periods were calculated for each individual rat as the performance measurement. Before the experiment started, the rats were tested so as to provide a baseline of the EMG activity. The EMG was normalized with respect to the baseline so as to eliminate the difference between subjects.

4.3 Results

The statistical significance of the weekly differences between the trained and control groups was evaluated by one way analysis of variance (ANOVA). The critical level for determination of significant difference was set as 0.05.

Figures. 4.21, 4.22, 4.23, 4.24 show the weekly comparison of the normalized mean EMG under different conditions (stimulation/rest) between the two groups. The results showed no significant difference between the two groups (tables 4.1, 4.2, 4.3 and 4.4 list the p-Values of the tests). This leads to the conclusion that daily training does not affect the response of the neural-muscular system to the loading/unloading perturbation. Or in other words, the EMG response depends more on each individual rat. This specific way of training couldn't override the spontaneous evolvement activities in the spinal cord after transection.

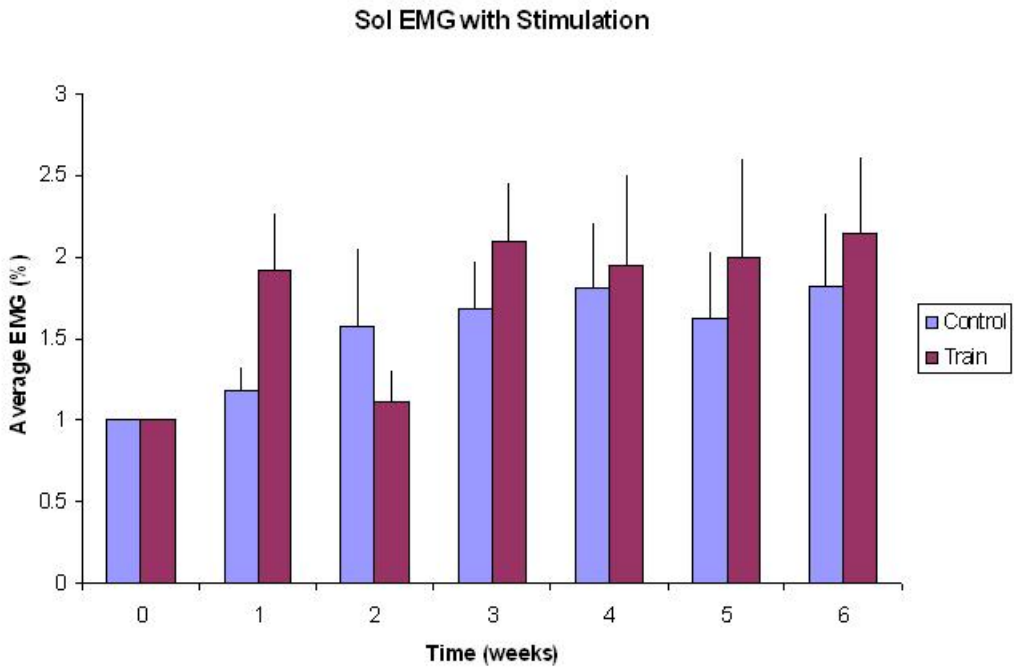

Figure 4.21: Weekly comparison of the average stimulation EMG (mean ± SE) in soleus between trained and control groups relative to the initial test session at week 0.

Table 4.2: The p-values of the ANOVA test of normalized rest EMG in soleus between trained and control groups

Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
0.1909	0.3705	0.2244	0.4718	0.304	0.7516

Table 4.3: The p-values of the ANOVA test of normalized simulation EMG in TA between trained and control groups

Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
0.1253	0.4617	0.053	0.0458	0.6664	0.7527

Table 4.4: The p-values of the ANOVA test of normalized rest EMG in TA between trained and control groups

Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
0.1241	0.247	0.4411	0.3928	0.753	0.4738

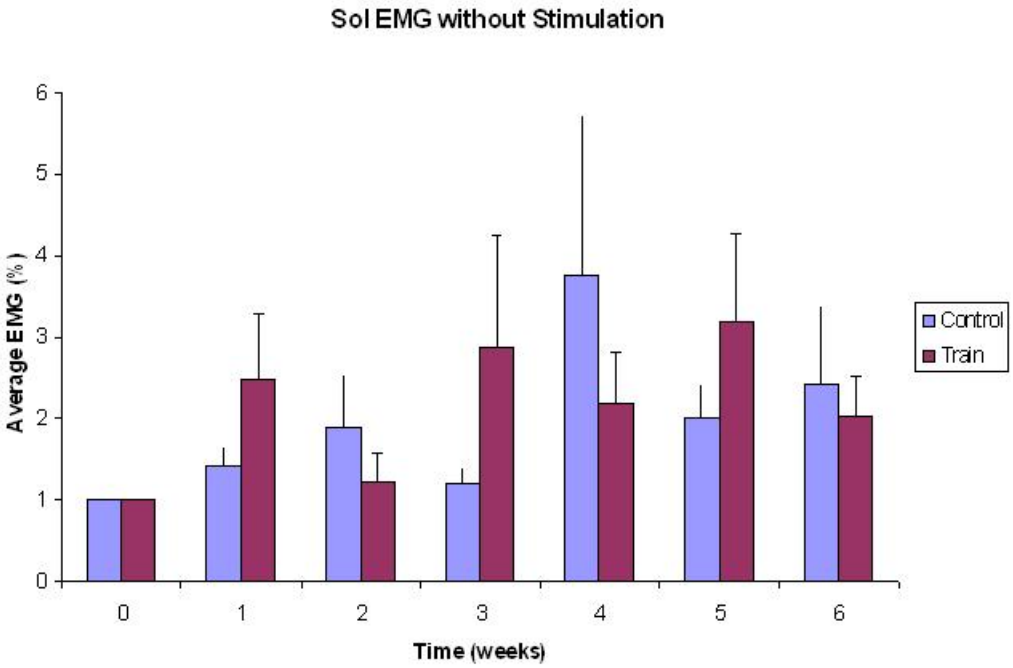

Figure 4.22: Weekly comparison of the average rest EMG (mean ± SE) in soleus between trained and control groups relative to the initial test session at week 0.

4.4 Discussion

The spinal cord has a great degree of automaticity and plasticity. After SCI, it can "learn" to perform a motor task such as stepping if it is intensively provided with the sensory information associated with stepping [1].

In the current experiment, we tested the ability of the spinal cord to adjust extensor responses when it is provided with everyday training of vertical perturbation. The mechanical perturbation triggered tonic supportive responses in the extensor muscles. However, no rats in the current experiment ever showed full weight bearing ability, i.e., they failed to generate enough muscle ability to support their body weight. Although the repetitive training can induce short term plasticity in motoneurons and lower the recruitment threshold [26, 27, 28]), it failed to have a long term effect facilitating motoneuron recruitment. This indicates that spinal cord has very limited ability in regulating the activity of the antigravity muscles, i.e., extensor muscles, when deprived of the descending tonic input from supraspinal nervous system. Also

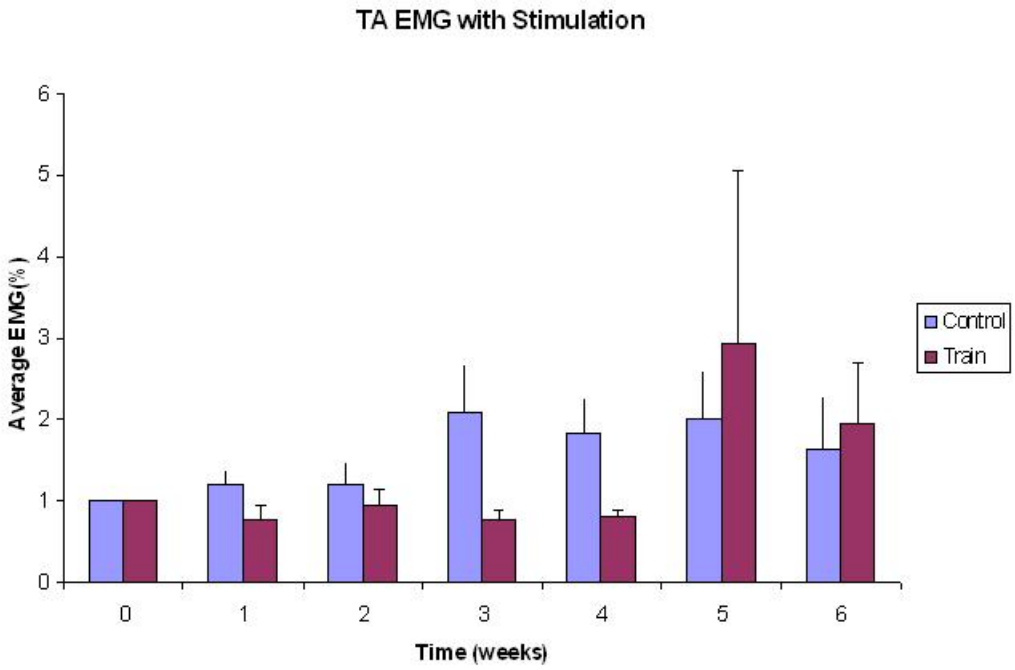

Figure 4.23: Weekly comparison of the average stimulation EMG (mean ± SE) in tibialis anterior between trained and control groups relative to the initial test session at week 0.

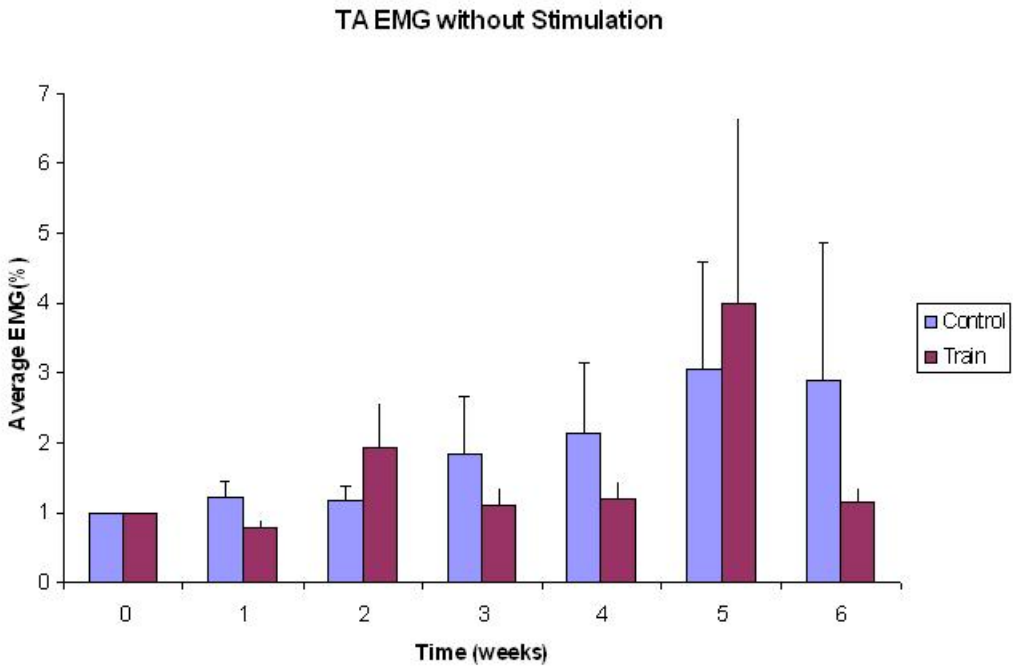

Figure 4.24: Weekly comparison of the average rest EMG (mean ± SE) in tibialis anterior between trained and control groups relative to the initial test session at week 0.

it was observed that the spinal rats had little active postural adjustment when the platform was tilted laterally, which indicates the spinal cord has very limited postural function when isolated from the higher central nervous system.

During the stance phase of stepping, spinal animals are able to generate a certain amount of body weight support and even full weight bearing [2, 31]. In this case, the motoneurons were activated by the phasic input from the local spinal cord circuitries (CPG) when appropriate sensory information was delivered into the spinal cord. In the current experiment, the platform gave vertical perturbation to generate load in the hind limbs trying to mimic the sensory input during standing. After a complete SCI, the rat cannot support its body weight so mechanical perturbation is needed to generate the sensory information of loading. The result of the current experiment showed that the mechanical perturbation failed to provoke sufficient tonic input to the motoneurons as required to perform weight bearing standing. Therefore, either the neuronal circuits regulating the antigravity muscle tone are not localized in the lumbosacral spinal cord or the mechanical perturbation is not enough to activate those circuits.

To explore other possibilities to generate tonic supportive activity, experiments in spinal rats were tried with spinal cord electrical stimulation on the first sacral (S1) spinal segment and with nonspecific activation by tail pinching (TP). Nonspecific activation by tail pinching produced tonic response during passive standing on a moving platform (figure 4.25). Low intensity pinching produced only tonic response (figure 4.25a) while more intensive pinching elicited rhythmic output (figure 4.25b). In both cases, TP filled the gaps between platform movements ($T = 4s$) and generated stable tonic responses (figure 4.25). ANOVA analysis indicates that the difference in the amplitude for tonic response before and after TP is greater than would be expected by chance for MG (p-Value = 0.026) and TA (p-Value = 0.001).

Spinal cord electrical stimulation ($40Hz$) of S1 spinal segment also facilitated the tonic response and produced longer duration responses, which effectively filled in the gaps between responses evoked by platform movements ($T = 4s$)(figure 4.26). The duration of tonic responses with and without SCS was $3.457 \pm 0.250s$ and $0.579 \pm$

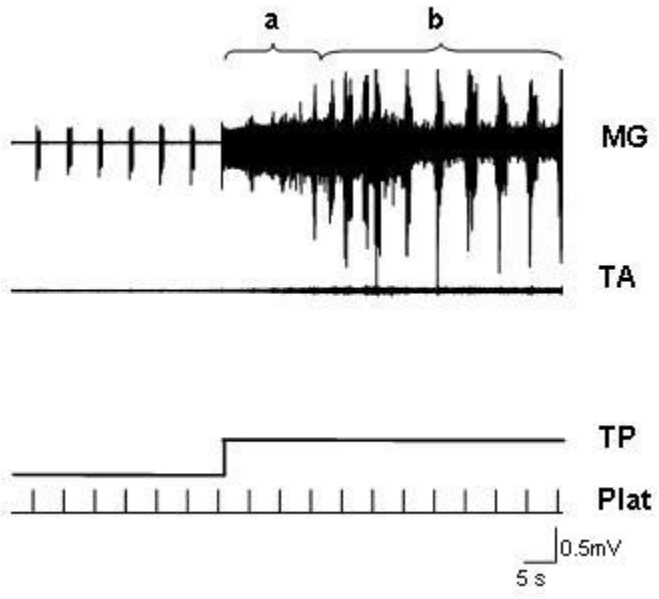

Figure 4.25: Effect of tail pinching (TP) on tonic response in spinal rats. TP increases the amplitude and duration of tonic responses in gastrocnemius(MG) and tibialis anterior (TA). (a)Low intensity pinching produced only tonic response; (b)more intensive pinching elicited rhythmic output.

$0.0343s$, respectively. The difference in the mean values of response from platform alone and from platform with SCS is greater than would be expected by chance (p-Value = 0.008). In comparison with TP, SCS did not change significantly the amplitude of tonic response (p-Value = 0.651). During the present study we usually observed the tonic response from SCS or TP mainly in the MG muscle, however some facilitation in TA muscles was also observed.

The maintenance of effective posture involves several spinal cord reflexes, including the stretch reflex and the crossed-extensor reflex. Inputs from sensory receptors of skin, joints and muscles provide information to the spinal cord circuits and also could contribute to balance control mediated by supraspinal mechanisms. A specific role for cutaneous receptors of the feet has been emphasized for precise balance control as well as tendon receptors that estimate joint angle [32, 33]. The role of muscles receptors in balance control is more complex due to the mechanisms for control of muscle stiffness. The main role of joint receptors is signaling the limits of joint excursion that are more important for complex movement such as locomotion rather standing.

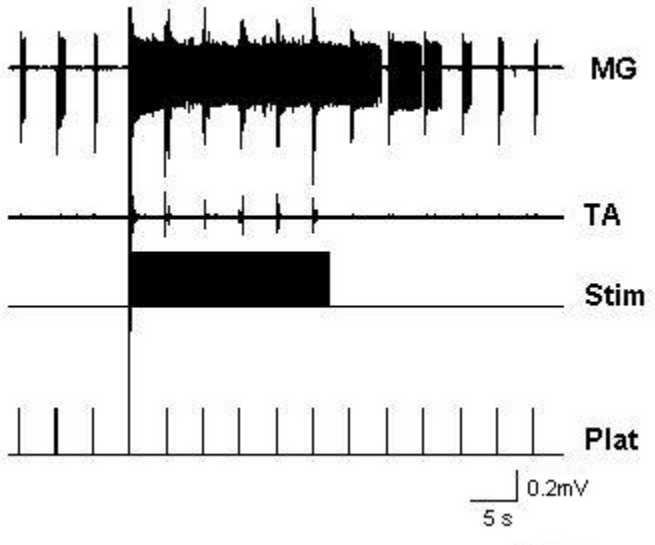

Figure 4.26: Effect of spinal cord stimulation (SCS) on tonic response in spinal rats. SCS enhances tonic responses in gastrocnemius (MG) and tibialis anterior (TA).

In our study activation of muscle receptors by platform movement produced tonic response. Effect of platform movement is presumably mediated mainly by activation of muscles receptors. Epidural stimulation that mainly activates afferents in dorsal column also facilitated tonic reaction; however the amplitude of the tonic response was not significantly higher for reaction on platform vibration and SCS. The largest excitatory effect we observed was from nonspecific activation of propriospinal system by pinching the tail.

After spinal cord injury neuronal circuits undergo several important changes. Immediately after transection, the sudden loss of tonic background provided by descending pathways results in a loss of muscle tone in the hind limbs. This period called spinal shock is characterized by the absence of spinal cord mono- and polysynaptic reflexes. After recovery from spinal shock, monosynaptic spinal cord reflexes recovered and were facilitated. Spinal polysynaptic reflexes exhibited some long-term recovery for several weeks after injury. Although recovery of spinal cord reflexes can be observed in spinal animals, the level of recovery was not enough to generate the tonic support needed for postural control after complete transection. The apparent lack of a postural equilibrium in the hind limbs of the chronic spinal animals suggests that

some of the activations of the neuronal circuits controlling postural stability may be located at higher levels than the spinal cord [34]. Although cats with a complete SCI at the lower thoracic region exhibit poor postural responses and as a rule are not able to maintain the dorsal-side-up orientation of their hindquarters [35], spinal cats can perform full weight bearing standing [15, 16, 35] and stand training does allow the spinal cat to stand for a longer period [3]. In contrast to cats, in rats the restoration of both standing and stepping did not occur after complete spinal transection. Although stepping in spinal rats can be improved by 5HT and NMDA agonists or by spinal cord stimulation there are no effective tools to enhance the tonic reactions in spinal rats. In the present study we did observe some tonic responses but this was not sufficient to make effective postural adjustment.

Based on the present data it can be concluded that after a complete transection those supportive tonic reactions can be mediated by several mechanisms: (1), activation of musculo-tendon mechanoreceptors by platform perturbation; (2), activation of afferents via spinal cord stimulation and (3), nonspecific tonic reaction to tail pinching. The mechanical perturbations only triggered reactions on the first level, which was not enough to support postural tasks such as fully bearing the body weight.

Finally, it can be concluded that it is not enough to purely use mechanical perturbation to generate the tonic responses needed to support the body weight. These studies suggest that some tonic support can be gained with spinal cord stimulation and mechanical stimulation of the tail.

4.5 Summary

In this chapter, experimental studies were conducted to test the potential of a newly developed robotic device to test and enhance the ability of a complete spinal rat to regain weight support in the hind limbs. Throughout 6 weeks of daily training, trained rats exhibited responses similar to untrained rats. This leads to the conclusion that without sufficient tonic input, the extensor response is not affected by the daily training and the spinal cord is barely able to generate any extensor response that is

enough for full weight bearing. To further study the postural functions of the spinal cord, other methods to generate tonic excitatory input should be involved.

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Chapter 5

Concluding Remarks

5.1 Thesis Summary

The first part of this thesis studied the effect of variability on the rehabilitation of stepping ability in adult spinal mice. We developed novel training algorithms that allow more freedom during training than the original fixed-trajectory training paradigm. Two assist-as-needed (AAN) algorithms were implemented and comparative experiments were conducted. The quantitative result showed that an AAN training paradigm with some level of imposed interlimb coordination had better training effect than the fixed-trajectory training and an AAN paradigm without interlimb coordination. This suggested that poorly designed training paradigms could hinder the recovery of stepping after SCI. More effective training should allow some variability so that more appropriate ensembles of sensory information could be generated and more natural motor activity could be elicited during training. The versatility and quantifying ability of robotic devices allow further exploration of more optimal training paradigms. We believe that further study will provide more insights into the learning mechanism of the spinal cord and thus in return help to develop more effective rehabilitative therapy.

The second part of this thesis involved a pilot study on the chronic effect of daily stand training. Analogous to step training, we raised the hypothesis that sensory information associated specifically with standing could increase the weight support ability after an SCI. The results showed no difference between the trained and control

groups under the current training strategy, unlike the observation of improved standing ability after training in cats. Despite this difference between rats and cats, one direct conclusion from the current experiment could be that the current training paradigm might not be effective. The other is that the antigravity muscles are regulated by supraspinal nervous systems at least in rats. However, the stand platform was shown to have the capacity to study the stand and postural response after an SCI. It can be used in cases of postural response study other than complete SCI. The results of chapter 4 could also be used as a baseline for comparison of future studies that used additional methods, such as SCS, to provide sufficient tonic input to replace the role of descending inputs.

5.2 Future Work

In step training, there is a huge parameter space for the training strategy. To find an optimal training paradigm obviously more studies are needed. This will be very demanding to do experimentally. Modeling and computer simulation may help to provide novel insight. Dr. Cai used machine learning theory to model the training process during the step training [1]. Although the model was simple, it show that a reenforcement learning mechanism very likely lies behind the learning of the spinal cord. So obviously a future direction would be establishing more realistic models of the neuro-musculo-skeleton system. The most difficult part would be the modeling of the neural circuits in the spinal cord, due to the complexity of the mechanism behind the interaction of neurons as well as complex structure of the undefined neural circuits.

The daily stand training of spinal rats did not affect the extensor responses as we expected based on stepping. Combined with observations from other studies when tonic input (spinal cord electrical stimulation and tail pinching) to the spinal cord was provided, the result of the current study implies that the regulation of the antigravity muscle tone is not a localized function of the spinal cord circuits without supraspinal tonic input in contrast with stepping CPG. From an engineering point of view, since standing is very closely attached with postural balance which is a complex

task requiring extensive sensory feedback, it is reasonable to suggest that antigravity muscle tone is regulated by supraspinal postural control centers, which receives the sensory feedback needed to sustain an upright posture.

It seems that tonic input should be provided to the spinal cord to facilitate a tonic response to support the body weight. Spinal cord electrical stimulation is one of the most direct methods available. Also pharmacological intervention can be used to adjust the biochemical environment in the spinal cord. Spinal cord stimulation is more direct than pharmacological intervention and it is faster in terms of response. Fine electrodes could be implanted to stimulate the appropriate motor neuron pools to activate appropriate muscles. Another possibility is that electrical stimulation, combined with postural feedback, may be able to control the balance in addition to simply support the body weight. However, many technical challenges such as bio-compatibility, and chronic consistency of the electrode location remain. Efforts in different disciplines are needed before stand rehabilitation after SCI can be applied clinically.

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